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PHYSICO-CHEMICAL FACTORS AFFECTING INTRA-OCULAR PRESSURE

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Until comparatively recent years physiological thought upon the problems of the intra-ocular pressure has centred itself largely round the blood pressure. It has long been known that the pressure in the eye varies directly with the pressure in the intra-ocular capillaries, a fact which recent work has merely confirmed. It has now become recognized, however, that an understanding of the problem of the maintenance and variations of the intra-ocular pressure involves also a consideration of the factors governing the production and elimination of the intra-ocular fluid as well as those influencing the volume of the vitreous body, for with any variations in these two humours (the only constituents of the globe that are subject to gross variation) the volume pressure of the eye must be considerably influenced.

The nature of the intra-ocular fluid. The earliest conception of the nature of the intra-ocular fluid was that it represented a secretion by the epithelium of the ciliary body, a conception based on insecure and inadequate evidence, mainly anatomical in nature. During the last 30 years there has existed alongside this view the theory that the fluid in the anterior chamber was formed by simple filtration from the capillaries of the eye. Later physico-chemical researches, however, have shown such a supposition to be inadequate to explain the formation of a fluid of its composition, and, largely upon the physico-chemical evidence which has recently accumulated, the theory that this fluid is essentially a dialysate has been founded. It is necessary therefore, in the first place, to consider this aspect of the problem.

An immense amount of work has been done on the chemistry of the intra-ocular fluids. Partial analyses involving the determination of the quantities of water, organic materials, total salts, etc., are to be found

in the writing of many authors since the time of Berzelius (1). More complete analyses have been made by Cahn (2), Lohmeyer (3), Mes-

TABLE 1

The general chemical composition of the intra-ocular fluids of the horse (Duke-Elder)

Quantities in grams per 100 cc.

	AQUEOUS	VITREOUS	SERUM
Water	99.6921	99.6813	93.3238
Solids dried at 100°C	1.0869	1.1087	9.5362
Total protein	0.0201	0.0662	7.3692
Albumin	0.0078	0.0077	2.9557
Globulin	0.0123	0.0115	4.4135
Mucoprotein	–	0.0211	–
"Residual protein"	–	0.0250	–
Fibrinogen	traces	traces	+
Immune bodies	traces	traces	+
Ferments	traces	traces	+
"Fats"	0.004	0.007	0.13
Cholesterol	traces?	0.0005	present
Non-protein N	0.0236	0.0264	0.0239
Total N	0.0269	0.0301	–
Urea	0.028	0.029	0.027
Amino acids	0.029	0.030	0.035
Creatinine	0.002	0.001	0.002
Organic acids,	traces	traces	–
"Sugar"	0.0983	0.0973	0.0910
Sodium	0.2787	0.2731	0.3351
Potassium	0.0189	0.0192	0.0201
Calcium	0.0062	0.0068	0.0101
Magnesium	0.0026	0.0020	0.0028
Chlorine	0.4371	0.4168	0.3664
Inorg. P (P_2O_5)	0.0033	0.0031	0.0030
Inorg. S (SO_4)	0.0061	0.0062	0.0058
Ammonia	0.003	–	Mestrezat and Magitot (4)
Lactic acid	0.02	–	Wittgenstein and Gaedertz (7)
Oxygen	20–40 mm.	–	de Haan (8), Mawas and Vincent (9)
	70 vols. %	–	
Total CO_2	71 vols. %	–	Takahashi (10) Kronfeld (11)
	60–70 vols. %	–	
	47.5 vols. %	43.7 vols. %	59.1 vols. % (Salit (12))

trezat and Magitot (4), Tron (5) and Duke-Elder (6). The general chemical composition of the intra-ocular fluid of the horse is given in table 1: with it are compared the composition of the vitreous body and the blood-serum. The figures are those of Duke-Elder (6) unless where otherwise stated.

The table shows that all the constituents of the serum are present in the intra-ocular fluid; but, when their relative concentrations are compared, it is seen that these constituents may be divided into three groups of substances depending on the physical state of their molecules in solution—colloids, non-ionized and ionized crystalloids.

Colloid substances. All the substances in colloidal aggregation are found in the intra-ocular fluid, but in much less concentration than in the serum—proteins, fats, immune bodies, and ferments.

TABLE 2

	COW		RABBIT	
	Intra-ocular fluid	Serum	Intra-ocular fluid	Serum
Total proteins	0.017	7.53	0.04	5.57
Globulin	0.009	3.73	0.009	1.16
Albumin	0.008	3.80	0.031	4.41
Quotient (Glob./Alb	50/50*	50/50*	20/80*	20/80*

* Approximate.

a. *Proteins.* All observers who have employed adequate methods are agreed that the normal intra-ocular fluid contains traces of protein. Many varying analytical methods have been used, both physical and chemical: among the physical methods are those depending on the refractive index (many authors), the viscosity (Scalinci, 13) and the surface tension (Dieter, 14); among the chemical methods, precipitation by Esbach's reagent (Wessely, 15, etc.), nitric acid (Gilbert, 16), trichloracetic acid (Mestrezat and Magitot, 4; Gala, 17, etc.), sulphosalicylic acid (Adler and Landis, 18), the micro-Kjeldahl method (Duke-Elder, 6), the nephelometer (Franceschetti and Wieland, 19), and so on. We may take it, then, that the presence of protein is definitely established. Not only in this case, but each type of protein is represented (albumin, globulin, fibrinogen), and they occur in the same relative proportion as it is met with in the serum.

Thus micro-Kjeldahl estimations gave the results seen in table 2 (Duke-Elder, 20).

Further it has been shown by a study of their reactions as anaphylactic antigens that these proteins are specifically the same as those found in the serum of the same animal (Duke-Elder, 20). It is seen, therefore, that although occurring in much less quantity, all the proteins of the plasma are found in the intra-ocular fluid, that they are found here in the same relative proportions in which they occur in the blood, and that they are specifically identical. They thus appear to remain unchanged in their transit from the blood-vessels to the eye.

b. *Immune substances.* Closely associated with the protein content of the intra-ocular fluid is the occurrence in it of the various substances connected with the chemical aspects of immunity. All recent work has gone to show that these bodies are invariably colloidal in nature; certainly it must be admitted that they are always associated with colloids, and that in all their activities they behave as such (see Duke-Elder, 20). The earlier writers held the view that there were few or no bacteriolytic substances in the intra-ocular fluid, and their absence was

TABLE 3

	INTRA-OCULAR FLUID		SERUM	
	Grams per 100 cc. sol.	Grams per 100 grams water	Grams per 100 cc. sol.	Grams per 100 grams water
Sugar	0.0983	0.0986	0.0910	0.0974
Urea	0.028	0.028	0.027	0.0289
Non-protein N	0.0236	0.0236	0.0239	0.0255

used to provide an argument in favour of the existence of a selective or secretory mechanism in the formation of this fluid. Since, however, they are colloidal it is to be expected that they will be found merely in traces, and recent work involving more refined methods of investigation has shown that they are present in the normal aqueous humour of highly immunized animals in much the same proportion as would be expected on purely physical grounds, in quantities closely corresponding with the concentration in proteins found there. (Cronstedt, 20; Gala and Fabian, 22; Franceschetti, 23, and others.)

Diffusible substances. These may be divided into two groups according to whether they are split up or not into electrically charged ions in solution.

a. *Non-dissociated diffusible substances.* It is found that the diffusible non-dissociated substances are partitioned between the intra-ocular fluid and the blood serum in approximately equal amounts. The more important constituents are seen in table 3 (Duke-Elder 20).

Sugar. Claude Bernard (24) was the first to identify "traces" of sugar in the aqueous humour of the ox. Subsequent observers obtained varying results: later and more accurate estimations have been recorded by Ask (25), Osborne (26), Holi (27), Mestrezat and Magitot (4), Hayano (28), de Haan and Creveld (8), Jess (29), Dieter (14), Cohen, Killian and Metzger (30), Tron (5), Ikebata (31), Yudkin, Krause, and their collaborators (32), and Adler (33). Ask (34) found an average in man of 0.10 gram per cent. The figures of Duke-Elder (20) bring out a close relationship between the intra-ocular fluid and the blood. They were obtained in the rabbit by the Hagedorn-Jensen method. The averages of the results are seen in table 4.

It is seen that the sugar content of venous plasma is considerably less than that of arterial plasma, and it is evident that that of the capillary plasma must lie between the two. The above analyses show that the sugar content of the intra-ocular fluid lies between that of the arterial and venous plasma and must therefore correspond closely with that of

TABLE 4

	GRAMS PER 100 CC. SOL.	GRAMS PER 100 GRAMS WATER
Intra-ocular fluid	0.151	0.151
Arterial plasma	0.148	0.158
Venous plasma	0.125	0.133

the capillary plasma. It should be noted that other observers have found the sugar content of the aqueous humour less than that of the blood (Ask, 36; de Haan and Creveld, 8; Adler, 33), a result which can be explained by the "binding" of a portion of the sugar to the proteins of the plasma, and the consumption of sugar in the eye by its tissues, especially the lens (Adler).

The other non-dissociated constituents are in a similar case. Andresen (35) found the urea in the aqueous sometimes above and sometimes below that in the plasma; and Rados (36) found, as is to be expected, the amino-acid content variable, but in concentration comparable to that in the blood: Pagani (37) gives the value of 22 to 40 mgm. per 100 cc. The concentration of lactic acid appears to be somewhat higher in the aqueous of some animals (dogs) though not in others (cat, rabbit), according to the micro-analysis of Wittgenstein and Gaedertz (7); but in all cases its quantity was found to vary intimately with that in the blood. These workers found 20 to 24 mgm. per cent in the aqueous and 15 to 18 mgm. per cent in the blood, while Fischer

(38) found a similarly higher percentage. The latter author brought out the very interesting fact that the concentration of lactic acid was much higher in the normal than in the aphakic eye (normal 20 to 28 mgm. per cent; aphakic 14 to 19 mgm. per cent): this points to the fact that the excess of lactic acid is partly due to glycolytic activity in the lens, and it must be remembered that the metabolism of the retina probably has a similar influence.

b. *Dissociated diffusible substances.* The dissociated diffusible substances are seen to be unequally distributed. In each case the cations have a partition co-efficient > 1, and the anions a partition co-efficient < 1, the former being in less and the latter in greater concentration

TABLE 5

	INTRA-OCULAR FLUID			SERUM		
	Grams	Per 100 cc. sol. Millimols per litre	Grams per 100 grams water	Grams	Per 100 cc. sol. Millimols per litre	Grams per 100 grams water
Cations:						
Sodium	0.2787	121.2	0.2795	0.3351	145.6	0.3585
Potassium	0.0189	4.8	0.0190	0.0201	5.1	0.0215
Calcium	0.0062	1.5	0.0063	0.0101	2.5	0.0108
Magnesium	0.0062	1.1	0.0026	0.0928	1.2	0.0030
Anions:						
Cl'	0.4371	123.1	0.4384	0.3664	103.2	0.3920
PO_4'''	0.0044	1.38	0.0044	0.0040	1.26	0.0043
SO_4''	0.0061	1.8	0.0062	0.0058	1.7	0.0062

in the intra-ocular fluid than in the serum. This is brought out clearly in table 5 (Duke-Elder, 20).

The figures in table 5 agree closely with other estimations in the literature: estimations of the metallic radicles (Na, K, Ca, Mg) are found in the writings of Cahn (2), Mestrezat and Magitot (4), Lebermann (39), Tron (5), and others. Estimations of the phosphates and sulphates, also in essential agreement, have been reported by Cahn (2), Mestrezat and Magitot (4), Heubner and Meyer-Bisch (40) and Tron (4). The chlorides, however, have attracted much attention, largely by reason of their greater concentration and the ease with which they can be determined. The literature commences with Berzelius (1); and the more recent and accurate determinations are found in Wessely (15) Lowenstein (41), Vladescu and Babes (42), Mestrezat and Magitot

(4), v. Creveld (43), Ascher (44), Rados (36), Jess (29), Gala (5, 17), Tron (5), Gaedertz and Wittgenstein (45). The values found in human aqueous humour are: Mestrezat and Magitot, 0.737 per cent NaCl; Rados, 0.879; Ascher, 0.7; Gala, 0.7. Duke-Elder's analysis (20) on the intra-ocular fluid of the rabbit, comparing it with the arterial and venous plasma of the same animals, gave the averages seen in table 6.

Of the *physical properties* of the intra-ocular fluid, the one which interests us most is the osmotic pressure. It has been determined cryoscopically (most recently by van der Hoeve, 46; Osborne, 47; Dieter, 14; and Colleati, 48), and by the method of plasmolysis, but the most accurate method devised is the direct method of measurement by a micro-osmometer (Duke-Elder, 49). The results showed that, owing to the excess of non-diffusible substances in it, the plasma exerts an osmotic pressure of about 20 to 30 mm. Hg greater than the intra-ocular fluid, the exact amount depending on the concentration of serum

TABLE 6

Chloride expressed as NaCl

	GRAMS PER 100 CC. SOL.	GRAMS PER 100 GRAMS WATER
Average of Intra-ocular fluid	0.635	0.637
Arterial plasma	0.579	0.619
Venous plasma	0.551	0.589

colloids. The osmotic concentration of the diffusible constituents of the intra-ocular fluid, on the other hand, lies between the point of equilibrium with arterial and venous blood, being much more closely related to the arterial: it is therefore in equilibrium with the osmotic concentration of the capillary plasma. The osmotic pressure of the intra-ocular fluid is therefore less than that of the capillary plasma by an amount determined by the excess of colloids in the latter. It is to be remembered, of course, that this difference is a small fraction only (about 0.3 to 0.5 per cent) of the total osmotic pressure (approximately 6,000 mm. Hg).

The reaction of the intra-ocular fluid. The intra-ocular fluid is slightly to the alkaline side of neutrality, being somewhat more acid than the blood, and having a pH from 7.1 to 7.3. Hertel (50), using the indicator method, was the first to measure the degree of alkalinity of the aqueous. He found a pH of 7.3 to 7.5 in man and 7.6 to 7.7 in the dog. Nordensen (51) obtained an average value of 7.5; Scalinci (52), 7.5 while arterial

blood was 7.4; Meesmann (53), 7.7 in man, the rabbit, and the dog, while the blood was 7.5; Baurmann (54), 7.43 to 7.52 arterial blood being 7.52 and venous 7.35; Schall (55) 7.5; Gala (17), 7.40 to 7.52; Mawas and Vincent (56), 7.55; Labbé and Lavagna (57), 7.5 to 7.6.

These values, however, were taken neglecting the very important factor of the CO_2 content of the fluid which is high (10 to 30 per cent higher than blood, Kronfeld, 58). Moreover, experiment shows that this gas is lost very readily indeed (Salit, 12). When this is taken into account the pH of the intra-ocular fluid is found to be less than that of the plasma—Baurmann (54) 7.2 to 7.35 (mean 7.268) when blood is 7.31; Kronfeld (58) 7.1 to 7.3. The fact that the aqueous humour is more acid than the blood is to be expected owing to the excess of acid radicles in it (Cl, etc.).

The pH value varies intimately with that of the blood (Meesmann, 53). Some authors consider that it varies with age; according to Nordensen (51) it is higher in the fetus than after birth, and Schall (55) considers that in later years it tends to become more acid. Salit (12) on the other hand, finds a tendency to increased alkalinity with age in cattle. It is probable that age has no influence *per se*, but the reaction varies simply with that of the blood.

Much information of considerable importance can be gained from a consideration of intra-ocular fluid formed under abnormal conditions. All the variations which take place in the composition of the intra-ocular fluid can be classed under one of two heads: alterations dependent upon a change in the permeability of the capillary walls, and alterations dependent upon a change in the composition of the blood.

A. *Alterations in the permeability of the capillary walls.* In conditions wherein the permeability of the capillary walls is increased very definite changes take place in the chemical and physical properties of the intra-ocular fluid, which are essentially the same no matter what method may be employed to alter the permeability—a mechanical lack of tone owing to a sudden fall of intra-ocular pressure, a vaso-dilatatory nervous reflex, or the direct action on the capillary walls of drugs or poisons whether introduced from without or liberated *in situ* by pathological processes. In a general sense it may be said that the fluid so formed differs from the normal by approximating more nearly to the constitution of the plasma: for this reason it may be called Plasmoid Intra-ocular Fluid. It is to be noted that this plasmoid fluid is found not only in the chambers of the eye, but also in its tissues, being evident as collections of protein-rich fluid under the ciliary epi-

thelium (Greeff's vesicles), in the iris, and in the sub-choroidal space. It has the following characteristics (see Duke-Elder, 20):

a. The *colloid* (*non-diffusible*) *substances are increased:* The proteins which in the normal fluid are present in traces only, are found in readily detectable quantities, and sufficient fibrin may be present to allow spontaneous coagulation. Cholesterol is also found, and immune bodies in considerable quantity, including precipitins which have not been detected in the normal fluid, while serological reactions which usually are not readily found in the normal aqueous humour are present in the plasmoid type of fluid—the Wassermann reaction, the gold-sol reaction, Pandy's reaction, and so on. Moreover, difficultly diffusing drugs which get into the normal aqueous humour only in traces, such as the organic compounds of arsenic, find their way readily into the plasmoid fluid.

b. The diffusible substances which are not ionized (such as sugar) are practically unchanged in concentration.

TABLE 7

	NORMAL INTRA-OCULAR FLUID	PLASMOID INTRA-OCULAR FLUID	DIFFERENCE PLASMOID NORMAL
Protein	0.04	2.5	+2.46
Sugar	0.165	0.172	+0.007
Chloride	0.500	0.421	−0.079

c. The ionized salts are partitioned unequally; the negatively-charged anions (chlorides, etc.) show a diminished, and the positively-charged cations (sodium, etc.) an increased concentration.

In a similar way the physical properties vary in the direction an excess of colloids would lead us to expect: the specific gravity increases, the refractive index increases, the surface tension approaches that of plasma, (Bardier and Cluzet, 59; Creveld, 60) the viscosity increases (Scalinci, 61; Mastrobuono, 62; Lowenstein, 41; Guglianetti, 63; Duke-Elder, 20) and as the concentration of protein increases the osmotic pressure becomes more nearly equal to that of blood, the diffusible constituents remaining all the time in equilibrium (Duke-Elder, 20).

The figures in table 7 from Duke-Elder's experiments on the rabbit (6) illustrate the characteristic increase in protein, the very slight increase in sugar, and the decrease in chlorides in percentages.

These relationships have been verified on repeated occasions in animals (bibliography, see Duke-Elder, 20), but in man the difference between

the intra-ocular fluid of new formation and the normal fluid is less marked. Some writers, indeed, suggest that there is no increase in the protein of the human plasmoid aqueous humour and that it therefore differs from that reformed in animals (Römer, 64; Hagen, 65; Gebb, 66; Rados, 36) but other workers, using more accurate methods of technique, have demonstrated that an increase of protein, although small, does occur (Mestrezat and Magitot, 4; Wessely, 15; Ascher, 44; Gilbert, 67; Gala, 17; Dieter, 14; Franceschetti and Weiland, 19; Samojloff, 68). Any difference between the two—man and animals—is therefore quantitative and not qualitative, and depends partly upon the fact that in the former the quantity of aqueous humour is relatively smaller so that its withdrawal involves a smaller capillary dilatation (Wessely, 69), but mainly upon the fact that the capillaries of the human eye are less permeable than those of some experimental animals.

From the clinical point of view most interest attaches to the increased permeability which is induced by the damage done to the capillary walls in inflammation. Here a typical plasmoid aqueous humour is formed. An increase of proteins in iritis and cyclitis has been demonstrated by Raehlmann (70), Schirmer (71), Löwenstein (72), Römer (64), Hagen (65), Gala (17), Gilbert (67), Magitot and D'Autrevaux (73), and Yudkin (74). A similar increase on sympathetic disturbance in the other eye was shown by Parisotti (75); and a decrease in salts was found by Gala (17) and Magitot and d'Autrevaux (73).

The increase of proteins in the plasmoid intra-ocular fluid after paracentesis has frequently been attributed to the increased difference of pressure suddenly produced between the capillaries and the chambers of the eye. That this is not the essential cause is seen in the formation of a plasmoid aqueous humour in conditions where capillary dilatation or increase of the permeability of their walls is brought about by factors which do not alter the pressure conditions appreciably. Further, when a maximal pressure disturbance is produced, as by paracentesis, and any capillary dilatation at the same time is prevented, a typical plasmoid aqueous does not form. This may be seen if a paracentesis is done after a retro-bulbar injection of adrenaline which has the effect of maintaining the capillaries in a state of relative constriction, when the secondary aqueous thus formed resembles the normal rather than the plasmoid type (Wessely, 15; Hagen, 65; Duke-Elder, 20). Similarly a non-plasmoid type is reformed after puncture with simultaneous stimulation of the sympathetic (Wessely, 15; Müller and Pflimlin, 76), or on puncture after ligation of the carotid (Wessely, 15; Magitot and

d'Autrevaux, 73). The composition of the aqueous is therefore a function of the state of capillary permeability rather than of the pressure difference between the blood stream and the chambers of the eye.

B. *Alterations in the constitution of the blood.* When the composition of the blood is altered either by varying the concentration of the normal constituents or by adding new substances to it, the intra-ocular fluids vary in the following way. The details are important from the point of view of the local availability of drugs in the eye.

a. *Colloid substances enter the eye only in traces, if at all.*

b. *Diffusible substances which are not ionized enter the eye freely,* rapidly attaining therein a concentration equal to that in the plasma.

c. Diffusible substances which are ionized behave in a manner depending upon their electrical charge. *Anions* (negatively charged substances, as chlorides) *readily pass into the eye;* but *cations* (positively charged substances as the metallic radicles) *do so only with difficulty.*

1. *Colloids.* It has been seen that colloids are present in the normal intra-ocular fluid in traces: on increasing the colloid content of the blood, this small representation in the eye may be proportionately increased; and on adding foreign substances of colloidal dimensions to the blood a similar small moiety may be discovered in the aqueous humour.

Thus on inducing artificial albuminuria in dogs, Vollaro (77) found that the albumen in the aqueous did not exceed the proportion obtained in normal dogs; Sugita (78) claimed to have obtained an increased quantity of cholesterol in the eye after feeding rabbits with lanolin; and in the case of those substances which are susceptible to tests of high delicacy, such as immune bodies, a slightly increased quantity can be detected in the aqueous when their concentration in the blood is greatly increased (Poleff, 79). Difficultly diffusible drugs, moreover, when they are injected into the blood stream, are found in traces in the normal aqueous. Thus the organic compounds of arsenic enter the eye only in negligible quantities (Löhlein, 80; Stühmer, 81; Neame and Bury, 82; Neame and Webster, 83; Krause, Yudkin and Morton 84). Bismuth acts in a similar way (Accardi, 85), and colloidal dyes such as trypan blue (Gaedertz and Wittgenstein, 45); but as we have seen in all these cases, when the permeability of the capillary walls is increased, as by a paracentesis, these large-moleculed substances can pass through in greater quantity.

2. *Non-ionized diffusible substances.* Substances on the other hand, which remain in a diffusible condition and carry no electric charge,

partition themselves according to the laws of diffusion equally between the intra-ocular fluid and the blood. Even substances in extremely minute concentration in the plasma can be detected in the aqueous when tests of sufficient delicacy are available for their identification, as witness adrenaline (Rossi, 86). The most important of such substances is sugar.

It has already been shown that the *concentration of sugar* in the capillary plasma and the intra-ocular fluid is strictly comparable. On the intravenous injection of sugar Duke-Elder has shown (87) that the concentration of this substance rises *pari passu* in the aqueous humour. A similar variation is seen in any condition wherein the sugar content of the blood is raised: the aqueous humour changes correspondingly, though slightly less quickly, while it returns to its normal condition somewhat more slowly than the blood. This is seen in diabetes (for more recent papers, see Ask, 25; Holi, 27; Dieter, 14) on the oral administration of sugar (Ask, 25; Hertel, 88; Holi, 27) on the injection of adrenaline (Calderano, 89; Holi, 27; de Haan and van Creveld, 43), and on extirpation of the pancreas (Calderano, 89). Conversely, it is decreased on the injection of insulin—Ohkuni (90). The sugar, therefore, in the intra-ocular fluid varies intimately and directly with that in the blood.

3. *Ionized diffusible substances.* The power of ionized diffusible substances to pass into the intra-ocular fluid from the blood depends upon the electric charge carried by the ion in question and upon the degree to which it is absorbed by the proteins of the plasma and becomes associated with these indiffusible bodies (Fischer, 38).

A. Diffusible *anions* invariably pass from the blood stream into the eye; this applies to acid dyes (fluorescine, eosin, orange G, chrysolin, eryocyanin, fuchsin S, aesculin, and others), to organic anions (salicylic acid, hydroferrocyanic acid, etc.), and to inorganic anions (iodide, bromide, chloride, etc.) (Gaedertz and Wittgenstein, 45).

Some of these associate themselves to a considerable extent with the plasma colloids and therefore are found in smaller quantities in the eye. An example of such is fluorescine. Thus Wessely (91) found that after its intravenous injection fluorescine was present in the blood in a concentration of 1/13,000, and in the aqueous humour of 1/1,000,000; Löhlein (92) found it also in corresponding proportions after its intravenous or subcutaneous injection. Nevertheless, it occurs in the intra-ocular fluid in the proportion which physical laws demand, a fact strikingly proved by de Haan and van Creveld (8) and Yoshida (93), who

showed that this dye appeared in the aqueous humour in the same concentration relative to the plasma as is obtained by dialysing blood through a collodion membrane.

Potassium ferrocyanide provides a good example of a diffusible substance in that it is non-toxic to animal tissues and the ferrocyanide anion is capable of easy recognition and quantitative estimation by the Berlin blue reaction. Its passage into the aqueous humour has been studied by many workers, notably by Löhlein (92), who found that its concentration in the aqueous and the blood described parallel curves, and that at all times its distribution between them was according to the physical laws of diffusion.

The *chloride* content of the aqueous humour has been shown repeatedly to vary with that of the plasma: it rises on the intravenous (Hertel, 88; Duke-Elder, 87), or the subconjunctival injection of salt (Wessely, 15; Verderame, 94; and Kochmann and Römer, 95). Similarly Gala (17) found that in patients with nephritis with chloride retention in the blood, the chloride of the aqueous humour was increased, and that it was similarly subnormal in pathological conditions when the blood chlorides were below the normal level. In the same way bromides administered by the mouth may be recovered in the intra-ocular fluid (Ascher, 44), or iodides whether administered orally, subcutaneously, subconjunctivally, or by skin inunction (see Löhlein, 92; Guglianetti, 63; and others).

B. *Diffusible cations*, on the other hand, do not readily find their way into the eye. This applies to both basic dyes (neutral red, methylene green, methylene blue, safronin O, brilliant cresyl blue, pyronin G, basic fuchsin, and others) and inorganic cations (sodium, potassium, calcium) (Gaedertz and Wittgenstein, 45). This law is of clinical importance with regard to the behaviour of metallic drugs, such as bismuth and mercury.

Based on these experimental data, the *theory of dialysation* was elaborated by Duke-Elder (20): that the intra-ocular fluid is in thermodynamical equilibrium with the capillary blood and is a dialysate of it, the dialysing membrane being the capillary walls. It is thus comparable in its origin and metabolism with the tissue fluids elsewhere, and differs from them qualitatively only as a consequence of the relative impermeability of the capillaries of the eye. The capillaries vary in their permeability throughout the different tissues of the body, being adapted to suit the needs of each particular organ, and their almost

complete impermeability in the eye may be regarded as a biological adaptation to keep the intra-ocular fluid as far as possible free from colloidal substances so that it remains optically homogeneous.

A consideration of the chemical constitution of the normal intra-ocular fluid shows that the terms of this equilibrium are strictly obeyed. No biological membrane is completely impermeable, and it has been seen that this fluid contains all the constituents of the plasma in the proportions in which we should expect to find them if we postulate a separating membrane relatively impermeable to the large-sized molecules. In the aqueous humour all the colloid materials of the plasma are represented merely by traces. The proteins, for example, are not new elaborations and show no evidence of chemical alteration: they are specifically identical with those of the plasma, they are all present, and they are found in the same relative proportions as they occur in the blood stream. Moreover, the proportions of these substances vary directly according to the simple laws of diffusion when the permeability of the membrane is varied.

These constituents which are freely diffusible, and which, not being ionized, are consequently not subjected to the electrical constraints imposed upon the system, are found in quantities which may be considered as equivalent when these are represented as concentrations in watery solution, *and when allowance is made for the influence of metabolic processes within the eye.* Thus substances such as sugar which are metabolised locally are in less concentration, while others such as urea and amino-acids, are found to vary considerably. Those constituents which, being ionized and carrying an electrical charge, are brought under the influence of these constraints, are found in the concentrations which theoretical considerations demand. Those anions which are entirely dialysable (chlorides) are found in the aqueous in higher proportions than in the blood, and the cations (sodium, calcium, etc.) which are retained on account of their electrical charge are found in the reverse ratio.

Thus Duke-Elder's analysis (20) showed that the relative concentrations expressed as normal chloride and sodium are:

$$Cl_{aq}:Cl_{blood} = 123:103 \text{ and } Na_{aq}:Na_{blood} = 121:145$$

The theoretical relation:

$$[Na^+]_{aq} \times [Cl^-]_{aq} = [Na^+]_{blood} \times [Cl^-]_{blood}.$$

becomes

$$121 \times 123 = 145 \times 103$$

or

$$148.83 = 149.35$$

an agreement which may be accepted as within biological limits.

Similarly Baurmann (96) estimating the chemical constitution of an ultra-filtrate of serum and the aqueous humour of rabbits, found them strictly comparable (table 8).

Further, we have seen that when the permeability of the membrane to colloids is increased, the plasmoid aqueous then formed, containing as it does a greater proportion of protein molecules, and therefore differing less profoundly from the plasma, and so involving the existence of less powerful stresses, changes its composition in terms of the thermodynamical postulates we are considering—the anions (chloride) decrease and the cations (sodium) increase *pari passu* with the increase in concentration of colloids, while the non-ionized substances (glucose) remain

TABLE 8

(*Results in milligrams per cent*)

	SERUM		SERUM ULTRA-FILTRATE		AQUEOUS HUMOUR
	Arterial	Venous	Arterial	Venous	
Na	300.3	302.2	313.9	315.7	316.9
K	24.44	22.1	23.2	21.44	23.3
Ca	16.2	15.7	10.5	10.5	10.6
Cl	402.6	401.9	431.0	427.1	434.6

unaltered. Again on introducing foreign substances into the blood-stream, these appear in the aqueous humour in corresponding concentrations; colloids are to all intents and purposes entirely retained in the blood, diffusible substances which are absorbed by the plasma proteins are partially retained, freely diffusible substances are partitioned in the ratio of their diffusion constants, and anions are allowed through while cations are held back.

From the chemical point of view, therefore, the intra-ocular fluid would appear to show both in its normal constitution and in the variations of its constitution from the normal, a complete membrane-equilibrium with the capillary blood, there being no evidence of the expenditure of any chemical energy in its formation, either in the elaboration of new substances, in the retention (apart from that on a physical basis) of substances already present, or in the abnormal concentration of any of its constituents (when the activities of local metabolism are allowed for).

2. *The osmotic-hydrostatic equilibrium.* Since the diffusible ions are unequally distributed a difference in osmotic pressure must exist between a dialysate and its parent fluid. We have seen that this difference obtains in fact, and that the osmotic pressure of the intra-ocular fluid corresponds exactly with that of a dialysate of the capillary blood both in the normal and abnormal state. Since there is thus a difference between the two osmotic pressures, equilibrium can only be obtained by a compensatory balancing in the hydrostatic pressures. It follows that if the system is in equilibrium, the blood pressure in the capillary circulation must exceed the hydrostatic pressure of the aqueous (i.e., the intra-ocular pressure) by an amount which is equal to the difference between the osmotic pressures of the two fluids. Thus equilibrium is attained when the hydrostatic flow from the blood stream to the eye is exactly compensated by an equal and opposite attraction from the intra-ocular fluid to the blood. It has been shown (Duke-Elder, 97) that no technique has been devised to measure the capillary pressure; but, having regard to the specialized anatomical and physiological conditions in the circulation of the eye, and reasoning from the unusual height of the arterial and venous pressures, reasons have been adduced for ascribing to the blood in the arterial end of the ocular capillaries a hydrostatic pressure rising to 25 or 30 mm. Hg above the intra-ocular pressure (Duke-Elder, 97). The conditions of the pressure equilibrium therefore seem to be fulfilled.

3. *The electrostatic equilibrium.* Lehmann and Meesmann (98) using a capillary electrometer and electrodes, one of which was introduced into the jugular vein and the other into the aqueous humour, found in cats, rabbits, and dogs, that a difference of the expected order (from 6 to 10 millivolts) existed between the two, the aqueous humour being positive and the blood negative. The relationship was upheld in abnormal conditions, for when the concentration of protein in the aqueous was made to rise, the difference in potential decreased, and by repeated punctures of the eye its value was made to sink to a few millivolts. Similarly in intra-ocular inflammation the potential difference fell; and by diminishing the colloid concentration of the blood by perfusion (in frogs) a similar fall was induced.

In addition, therefore, to the fact of the existence of a chemical equilibrium, all the conditions of a physical equilibrium—hydrostatic, osmotic and electrostatic—seem also to be fulfilled.

The evidence therefore points to the fact that the intra-ocular fluid is a dialysate of the capillary plasma because it has all the very definite

physico-chemical properties both in its normal and abnormal state which such an origin demands, because there is no evidence of the expenditure of any energy or demonstration of any activity in its formation beyond the stresses set up by the restraint placed upon the equal diffusion of colloidal molecules by the dialysing membrane, and because it is in chemical, hydrostatic, osmotic, and electrostatic equilibrium with the capillary blood. Such a physico-chemical method of enquiry is much more susceptible to adequate control and much less open to experimental fallacy than are the abnormal incidentals of physiological experiment or the equivocal deductions of histological technique upon "normal" or pathological structures. The capillary walls (and not the ciliary epithelium) must form the essential dialysing membrane, because the intra-ocular fluid is in equilibrium with the capillary blood, because its properties vary directly with the state of the capillary permeability, and because no localized region in the eye is the single site of the process of dialysation. On either side of the capillary walls there is a balancing hydrostatic and osmotic pressure equilibrium, around which level the mutually compensating pressures are continually fluctuating, and in any capillary these balancing forces will determine a flow of fluid outwards at one moment, and inwards at another. This fluid will percolate throughout all the structures within the sclera, through the stroma and cellular elements of the ocular tissues, and eventually through the epithelium and the endothelium lining the anterior and the posterior chambers, filling these cavities and percolating the substance of the vitreous. Throughout its passage, although it is modified by metabolic activity and the stresses to which it is exposed, no evidence of any expenditure of energy can be detected: but although the process seems to be a purely passive one, at the same time it must be remembered that at all points of its course, between each cell and between the interfaces in each cell, fresh membranes have to be traversed, each involving a new series of stresses with the corresponding adjustments that these entail, and each necessitating a fresh state of thermo-dynamical equilibrium.

When the permeability of the capillaries is increased certain very definite adjustments are necessitated in the thermo-dynamical equilibrium; but it is evident that, however produced, the plasmoid aqueous humour so formed always retains the characteristics of a dialysate. Its formation, therefore, corresponds to the local edema which may be produced in any region of the body when capillary dilatation has been brought about by a variety of means, as by mechanical or thermal stimulation, the action of drugs, or other agencies. When the pressure

of the eye is suddenly reduced, as in a paracentesis, the process of dialysation becomes temporarily converted into one of ultra-filtration. In these circumstances a plasmoid fluid is not only found in the chambers of the eye, but in the tissues as well, and since this colloid-rich fluid finds difficulty in dialysing through the cellular layers of the tissues owing to its complement of large molecules, it tends to collect in vesicles underneath the ciliary epithelium (Greeff, 99), on the posterior surface of the iris (Carlini 100; Carrére, 101; Samojloff, 102) and in the subchoroidal space as is seen in post-operative effusions (Collins, 103). The histological picture presented by these changes is one of a simple passive transudate (Henderson and Lane-Claypon, 104); and the clinical and physical nature of the fluid, in whatever circumstances it is formed and by whatever influences, is explicable in terms of a thermo-dynamical equilibrium.

The formation of the intra-ocular fluid by dialysation allows no impetus for a through-and-through circulation of fluid; and a large number of experimental and innumerable clinical observations (Duke-Elder, 20) show clearly that such a circulation does in fact exist. It is probable that such a circulation is determined by variations in pressure caused by the pulse beat, the respiratory rhythm, and muscular activity (Duke-Elder, 105). According to this view, not only the origin but the circulation of the intra-ocular fluids, falls into line with the tissue-fluids generally.

On an increment of pressure a small quantity of fluid is forced out of the eye, and the position of the canal of Schlemm, situated far down the venous pressure gradient, with its delicately adjusted pressure equilibrium will favour a preferential exit at the angle of the anterior chamber. It has been shown (Duke-Elder, 97) that in normal circumstances, since the pressure in the venous exits is higher than that in the chamber of the eye, a hydrostatic outflow is impossible. In conditions of raised pressure, however, the relations are reversed, and the canal of Schlemm, wherein the pressure is lowered temporarily below the chamber pressure, thus acts as a safety valve, while the action of the ciliary muscle upon the scleral spur will aid the outflow of fluid still further by suction. Similarly the tendency of the ciliary muscle to open out the choroidal veins on its contraction will favour the elimination of fluid in the posterior segment of the eye. In this way the contractions of the orbital musculature and of the ciliary muscle as well as the pulse beat, the respiratory excursion, and the constant movements of the lids will provide continuously changing pressure

conditions, and a virtually constant but minimal pressure circulation will thus be imposed upon the fluid contents of the globe.

The nature of the vitreous body. The chemical constitution of the vitreous body has already been given in table 1. From this table it is evident that it is made up of the constituents of the intra-ocular fluid (*hematogenous constituents*) in proportions comparable to those in which they occur therein, with the addition of two specific or *non-hematogenous* substances, the muco-protein and the residual protein. Chemically, therefore, the vitreous body may be regarded as a gel formed by a combination of the intra-ocular fluid with these two latter substances.

A considerable amount of work has been done upon the non-specific constituents of the vitreous body. The table gives the analyses of Duke-Elder (105). Other instructive papers are: Jess (29), Cohen, Killian and Metzger (30) and Tron (5). Papers on the hematogenous protein constituents are Ascher (44), Gebb (60), and Franceschetti and Wieland (19); on the sugar content, Ask (34); and on the salt content, Ascher (44), Lebermann (39), and Cohen, Killian, and Kamner (106).

It is evident from the table that those constituents of the vitreous body which are ultimately derived from the blood are present in proportions comparable to those found in the intra-ocular fluid. The colloid constituents of the blood are present in very minute proportions, the proteins being specifically identical with the serum proteins; the non-ionized constituents are present in comparable proportions; while, of the ionized constituents of the plasma, the cations are found in less concentration and the anions in greater. It appears, then, that these constituents of the vitreous body, like all the constituents of the aqueous humour, are formed by a simple process of dialysation from the capillary plasma.

The *special* (*non-hematogenous*) *constituents* of the vitreous body are two in number.

1. *Muco-protein.* A mucinous substance was detected in the vitreous body by Virchow (107), Portes (108), Giacosa (109), Boë (110), and Mörner (111). In its chemical nature it is a true mucin (Levene, 112) being precipitated by dilute acetic acid in excess, and reducing Fehling's solution on hydrolysis. Mucoitin sulphuric acid B can be prepared from it by suitably suspending the lead salt in water and liberating the cation by acid; chitosamine is present (16.66 per cent) and glucuronic acid (23 per cent). Such a material is found in the umbilical cord and the cornea in addition to the vitreous, and it is possible that it has a definite optical function—that of the maintenance of transparency.

2. *The residual protein.* The material which is separated from the vitreous gel by filtration appears as a sticky, gummy material, which after washing and drying resembles a dry and horny piece of gelatin. It does not dissolve in water, but is extremely hygroscopic; it swells up into a clear gel to a slight degree in acids, and to a very marked degree in alkalis, and it completely and readily dissolves on boiling with either acids or alkalis. It contains no carbohydrate radicles. Its chemical nature has not yet been elucidated, nor is it yet certain that it is represented by a single chemical substance. Considered as such its elementary composition is as follows (Duke-Elder, 113):

	per cent
C	44.46
H	6.415
N	12.20
Ash	3.825
Including:	
P	0.12
S	0.675

Its titration curve shows its protein nature and demonstrates that it combines with acids and alkalis under appropriate conditions (Duke-Elder, 113).

The importance of this substance appears to be its power of forming a gel. If the proteins of the whole vitreous are coagulated by treatment with alcohol and extraction with ether at −5°C., a mass of gel can be obtained (an artificial vitreous) by the simple addition of water; if, however, the proteins of a filtrate of the vitreous (that is, in the absence of this constituent) are similarly treated, gel formation seems impossible. The extreme dilution in which this substance is capable of gel formation in vitro is especially remarkable (0.025 per cent); and it would, therefore, seem that, like the muco-protein, this unknown constituent (or group of constituents) has a very special function.

The abnormal vitreous body. It has already been shown that when the permeability of the capillaries is increased the composition of the aqueous humour changes in terms of a thermo-dynamical equilibrium: the concentration of serum proteins is increased, and at the same time the cations increase, and the anions decrease by an amount required by such an equilibrium, while the undissociated diffusible constituents remain relatively unchanged. Experiment shows that similar changes can be observed in the vitreous body in similar circumstances (Duke-Elder, 113). The reservation must be made, however, that the changes are

smaller and take a longer time to attain a maximum and a considerably longer time to return to normal, a fact which follows logically from the difficulty which will be experienced by colloids in diffusing into the gel.

Anatomically the vitreous body in the fresh state is now known to be a gel without structure in the usual anatomical sense (Duke-Elder, 113); ultra-microscopically it is found to contain linear micellae, resembling somewhat a solution of soap (Baurmann, 114; Komberg, 115; Heesch, 116; Duke-Elder, 113). Embryologically it has been shown to be derived from the surrounding ectodermal tissues, particularly the retina. Its chemical properties now make it clear that it is a simple gel, formed on the basis of two special protein constituents (the muco-protein and the residual protein) and that the common intra-ocular fluid which dialyses from the capillary blood percolates these, combining with them physically to form the gel.

The vitreous gel is not a unique form of inorganic matter different from other forms of matter found in nature. If blood-plasma is diluted 50 times with saline and allowed to clot by inoculation with a trace of normal serum, a gel of closely similar characteristics results (Duke-Elder and Robertson, 118). Its vapour pressure isotherm shows that it is a reversible elastic gel (Duke-Elder 113), and the measurement of its elasticity confirms its close relationship in structure with simple gels as gelatine (Duke-Elder and Robertson, 118).

From the point of view of the consideration of the intra-ocular pressure the most important characteristic of the gel is its capacity for turgescence and deturgescence. The turgescence curve of the pure vitreous proteins as a whole can be analyzed as follows (Duke-Elder, 113).

1. An acid zone (pH 1 to 4) where turgescence attains a maximum (at about pH 3.5), the weight of the vitreous increasing at this point 42.55 times. From this maximum the curve falls even more sharply than it had risen.

2. The iso-electric zone (pH 4 to 6), where the curve continues to fall sharply until a minimum is reached in the region of pH 4.5 (the iso-electric point); thereafter it rises again.

3. The neutral zone (pH 6 to 8), in which the curve continues to rise, but much more slowly, until a second maximum somewhat lower than the first is reached just short of pH 8.

4. A zone of instability (pH 8 to 9), in which the curve is found to vary somewhat; the degree of turgescence may be found to increase still further after pH 8, but in the majority of cases it falls considerably,

reaching a second but less accentuated minimum between pH 8.2 and pH 8.3. Thereafter it rises again rapidly to the height it had previously attained in the region of pH 8.

5. In the highly alkaline zone above pH 9, the curve of turgescence shows a maximum at about pH 9.5, whereafter it shows a sudden and sharp fall.

When salts are added the variations in turgescence are seen to follow in general terms the changes which occur with the pure acid or alkaline solutions, except that the irregularities of the curve are, as it were, equalized out. The acid rise is inhibited; the fall of turgescence in the region of the iso-electric point becomes less acute; the alkaline rise is also inhibited but to a considerably less extent; and the fall in the higher degrees of alkalinity is brought on more rapidly.

Of more immediate interest is the behaviour of the entire gel mass under varying conditions, since, owing to the small distensibility of the sclera, any change in gel volume will be reflected in a variation of the intra-ocular pressure. With this end in view measurements of the volume of the vitreous after bringing it into contact with solutions of various pH were recorded by Baurmann (119), Duke-Elder (113), and Lobeck (120). Baurmann (119) concluded that in the normal state the vitreous gel was in a condition of maximum turgescence; Lobeck (120) obtained indeterminate results, a circumstance which seemed to depend upon the apparatus employed. Duke-Elder (113) found a minimum volume (iso-electric point) between pH 4 and 5, at which point the condition of the gel is changed by the appearance in it of a coagulated felt-work of white opaque fibres. Thereafter the volume rises until pH 8 is reached, beyond which point, between pH 8 and 9 a zone of instability occurs; beyond this an increase of volume occurs, until pH 10, when the volume is decreased to a fraction of the original, and the mass of gel again becomes interlaced with a felting of fine opaque fibres, resembling those which appear in the very acid solution.

The maintenance of the intra-ocular pressure. The normal intra-ocular pressure may be taken to be that evolved as the optimum at which the eye is rendered optically rigid and at which its circulation and the metabolism of its tissues can at the same time proceed without disturbance. The eye may be looked upon as a chamber containing fluid enclosed within a feebly distensible envelope formed by the sclera and cornea. The pressure within is maintained at its normal height by two factors—the elasticity of the outer coat and the volume of its contents which is maintained by the energy derived from the blood pressure.

Experiment shows that when an eye is excised or exsanguinated it retains an internal pressure of about 10 mm. Hg (Leber, 121; Magitot, 122; Duke-Elder, 123), at which level it remains until post-mortem disintegration of its tissues sets in, when the pressure gradually falls to the atmospheric level: this amount of residual tension is therefore maintained by the elasticity of the sclera compressing the contents of the globe. On allowing the flow of blood to resume in the exsanguinated eye, as, for example, on ligating and then freeing the carotid, or on exsanguination and subsequent perfusion, the vascular channels are refilled, and, since the external coat is only feebly distensible, the entrance of additional fluid involves a rise of pressure. In this way the feeding arteries pile up pressure, and the intra-ocular pressure therefore rises until a point is reached when an adequate circulation is maintained. At this point the pressure equilibrium is established, and the ultimate height of the pressure in the chambers of the eye is determined by the hydrostatic pressure in the capillaries minus the difference in osmotic pressure between the aqueous humour and capillary plasma. If we put the lateral component of the hydrostatic pressure in the capillaries at about 50 mm. Hg (Duke-Elder, 97), and the difference between the osmotic pressures of the two fluids at 30 mm. Hg, the resultant pressure in the chambers of the eye will be 20 mm. Hg.

To a very large extent this pressure level is maintained relatively constant in the normal eye by the safety-valve mechanism of the canal of Schlemm. Owing to the poor distensibility of the sclera any changes in the volume of the contents of the globe are immediately reflected in marked changes in pressure. The pressure equilibrium in the region of the canal of Schlemm has already been dealt with when it was pointed out that normally the venous pressure in the exit veins was higher than the intra-ocular pressure; in conditions of raised tension, however, this relation is reversed and an outflow of fluid was thus rendered possible (Duke-Elder, 97). Any outflow of this nature will immediately lower the internal pressure, and by means of this safety-valve action the aqueous humour will thus act as an elastic cushion, tending within limits to maintain the chamber-pressure at its normal level. If, however, the exit channels are rendered inefficient, a rise in pressure will result at an early stage. Moreover, if the changes are great, not only may the aqueous humour be pushed out, but the uveal blood channels may be compressed, in which case the feeding arteries will pile up pressure to maintain the circulation at a higher level, until eventually their effective pressure may be reached and finally overcome; in the

first case the tension will be permanently raised, in the second the eye will be strangulated.

The variation of the intra-ocular pressure. The intra-ocular pressure may be varied from its normal height by three groups of factors.

I. External pressure upon the globe, which in ordinary circumstances depends upon the action of the extra-ocular musculature.

II. By altering the equilibrium level.

a. By raising or lowering the pressure in the capillaries.

b. By varying the difference between the osmotic pressure of the capillary plasma and the intra-ocular fluid.

III. By varying the volume pressure inside the eye, which variation is rendered effective by virtue of the small degree of distention of which the sclera is capable.

1. By varying the volume of the contents of the globe.

a. By varying the state of dilatation of the uveal capillaries.

b. By varying the quantity of the intra-ocular fluid.

c. By varying the volume occupied by the vitreous and lens.

2. By varying the volume capacity of the globe; by inducing a turgescence of the sclera.

Of these influences the action of the external muscle of the globe has no physico-chemical interest: the subject will be found discussed in Duke-Elder (124). Variation of pressure in the capillaries has long been known to influence the intra-ocular pressure; the only new fact which has emerged of recent years is the great importance of the action of axon-reflexes in causing acute rises in the pressure of the eye by virtue of the occurrence over the entire uveal tract of a typical "triple response," a phenomenon which, among all internal organs, appears to be peculiar to the eye (Duke-Elder, 125).

With regard to variations in the equilibrium level by osmotic changes, considerable interest arises. Since the capillaries are freely permeable to crystalloids, which therefore distribute themselves in equivalent proportions between the intra-ocular fluid and the plasma, the difference in osmotic pressure between these two fluids can only be altered for any length of time by varying the relative concentration of their colloid constituents. If the colloids in the blood are increased the difference between the osmotic pressures of the aqueous humour and the plasma (which difference, as we have seen, depends upon their relative colloid content) is correspondingly increased; the intra-ocular pressure, the level of which is determined by the capillary pressure less this difference, therefore falls. Conversely, if the colloid content of the blood is de-

creased, its osmotic pressure approaches more nearly that of the intra-ocular fluid, and the intra-ocular pressure approaches more nearly that of the hydrostatic level in the capillaries. These changes have all been demonstrated experimentally (Duke-Elder, 126).

The intra-ocular fluid contains practically no colloids, so that any change must involve an increase of its colloid content. Such a change will make the two osmotic pressures approximate, so that the chamber pressure in the eye must rise. This may be shown experimentally if the colloid content of the aqueous is increased by partially replacing the normal fluid under constant pressure conditions by a colloid rich fluid (Duke-Elder, 126); and its clinical counterpart is evident in the rise of pressure which occurs in all cases when a plasmoid aqueous is formed. This is sometimes particularly evident when the protein content of the intra-ocular fluid becomes high in iritis.

Changes in the volume pressure. Since the distensibility of the envelope of the eye is very small the intra-ocular pressure may be varied by altering the volume occupied by any of its contents. Thus dilatation of the capillaries brings about a large rise of pressure, and their contraction a corresponding fall.

Alteration in the volume of the intra-ocular fluid. In the normal state the fluid traffic of the body is regulated essentially by the equilibrium between the amount driven out of the circulation into the tissue spaces by filtration due to the blood pressure, and that transferred back into the blood stream by the effective osmotic pressure of the blood over the tissue-fluid, due to the colloids of the former. If the molecular concentration of the blood is raised, since an effective increase over filtration pressure is thus established, fluid is transferred in excess from the tissues into the blood, dehydrating the former, and increasing the volume and pressure of the latter. As salt equilibrium is quickly re-established, the effect is only temporary, but at the same time the lessening of osmotic resistance to filtration in the kidney by dilution of the colloids induces a great diuresis and thus intensifies the general state of dehydration. Converse considerations are applicable if the molecular concentration of the blood is lowered.

It follows that if anisotonic solutions (e.g., salt, sugar) be injected into the blood stream, the eye shares in the general state of osmotic hydremia or depletion which the injection induces over the whole body, and since the volume of fluid in the eye is altered, changes in the intra-ocular pressure are brought about (Cantonnet, 127; Hertel, 88; Haupt, 128; Sansum, 129; Pletnewa, 130; Weekers, 131; Marx, 132; Duke-Elder,

133; Fremont-Smith and Forbes, 134; Ogawa, 135). Thus on the intravenous injection of hypertonic saline, after a preliminary variation with the blood pressure, the intra-ocular pressure rapidly falls, and remains at a low level for some considerable time. Conversely, on the injection of hypotonic solutions into the blood stream, the whole body including the eye becomes waterlogged, and the intra-ocular pressure rises, again independently of the blood pressure. That this pressure variation depends in large measure on the change in the quantity of the intra-ocular fluids can be shown by estimating the quantity of water by weighing the eyes before and after drying at a high temperature, in one eye before the experiment is commenced, and in the other eye after the injection (Hertel, 88; Duke-Elder, 133).

Variations in the volume of the vitreous body. We have seen that the vitreous body is a hydrophilic gel capable of adsorbing water and of undergoing changes of turgescence and deturgescence. We have also seen that, in the neighbourhood of its normal reaction, an increase of alkalinity will tend to make the gel swell, and an increase of acidity will tend to make it shrink; and further, that an increase in the salt content of the fluid bathing it will tend to damp down either of these changes. These factors can be shown to influence the intra-ocular pressure experimentally (Duke-Elder, 113, 136): a fall of pressure is obtained in the perfused eye by lowering the pH of the blood, and a rise is produced by raising the pH.

How much these changes affect the intra-ocular pressure *in vivo* (a question first raised by Fischer, 137) is not easily answered. The constancy with which the reaction of the blood remains at the normal level despite attempts to change it is remarkable, and any attempt to cause gross changes is incompatible with life (Nakamura, 138). The results in the literature of investigations into this question are somewhat contradictory: Meesmann (53) obtained a diminution of pressure on injecting the eye with isotonic solutions of a low pH, and an increased pressure on using solutions of high alkalinity. He verified these results by clinical observations on glaucomatous patients, and his results were confirmed by Redslob (139). In contradistinction to these Oguchi (140) found an increase of pressure on injecting both acid and alkaline solutions into the eye, and Dominguez (141) arrived at the same conclusion in the enucleated eye. Again, Schmelzer (142) and Wegner and Endres (143), after raising the pH of the blood by over-ventilation of the lungs and by the exhibition of large quantities of sodium bicarbonate, failed to find any alteration in the pressure of the eye. Moreover, a very large

number of observers have investigated the reaction of the blood or of the intra-ocular fluids in glaucomatous patients, and on comparing them with the normal have found little or no difference. The evidence would seem to point to the fact that while a swelling and shrinkage of the vitreous can be demonstrated experimentally to follow upon a change in the reaction of the blood if sufficient precautions are taken in the technique, yet these changes are of questionable clinical importance. At the same time, it is almost certainly the case that alterations in volume of the vitreous body, brought on presumably by changes in its metabolism which have not yet been elucidated, do occur and do profoundly influence the pressure of the eye.

About *changes in volume of the lens* we know little. The iso-electric point of the lens' proteins is at a pH lower than that of the normal reaction, and therefore we would expect it to swell in alkaline media and shrink in acid media. Moreover, acting as it does as an osmometer, it undergoes changes in volume when the osmotic equilibrium between it and the surrounding fluids is altered: this may be responsible for changes in its refracting power (for example, in diabetes) (Duke-Elder, 144). It is questionable, however, if such changes ever influence the pressure of the eye to any great extent, for they can only develop slowly and must easily be accommodated by a corresponding expulsion of intra-ocular fluid. In glaucomatous states, however, there is evidence that a swollen lens may intensify the effect of a swollen vitreous body, partly through purely volumetric considerations and partly by embarrassing the filtration channels of the eye by crowding up the circumlental space and the angle of the anterior chamber.

Changes in the volume capacity of the eye can be made to influence the intra-ocular pressure profoundly in experimental conditions. It has been found that the sclera turgesces considerably in acid and slightly in alkaline solutions, and in this event will considerably diminish the space left for the contents of the globe. The classical experiments of Fischer (137) who found that on immersing the eye in acid solutions a pressure could be generated of such a degree that the eye burst, are probably explicable largely by this factor. Fischer himself attributed his results to a swelling of the vitreous body, but the work of v. Fürth and Hanke (145), v. Ruben (146), Nakamura (138) and Heesch (116), put it beyond doubt that a swelling of the sclera with the diminution of the volume of the eye which it entailed, was sufficient to account for the changes observed. It is to be remembered, however, that the concentrations of acid which must be used to produce these effects are far

above any which can occur within the limits of variation compatible with life, and any effect which such a mechanism may have clinically must be looked upon with great reserve.

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THE INNERVATION OF SKELETAL MUSCLE

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The earlier literature bearing on the innervation of skeletal muscle is brought together in the review by Regaud and Favre (1904). Starting with the paper of J. T. Wilson (1921), a number of treatises have dealt in detail with the literature of certain problems of muscle innervation, i.e., Cobb (1925), Needham (1926), Fulton (1926, Chap. xvi), Hines (1927, 1930), Hinsey (1927, 1928, 1930), Ranson (1929), Forbes (1929), Gasser (1930), Bremer (1932), Cobb and Wolff (1932) and Creed, Denny-Brown, Eccles, Liddell and Sherrington (1932). The dominant questions in all of these have been, "What is the anatomical substratum for postural contraction (muscle tonus) and how is it activated?" Here, as in other phenomena of biological interest, it is very difficult to separate the morphology of skeletal muscle and its nerve supply from its function. In this review, we shall endeavor to present the anatomical evidence and shall refer from time to time only to some of the significant physiological observations bearing upon the problem.

Histological methods. There are three methods which have been used extensively in this problem: 1, gold chloride (Löwit, 1875; Ranvier, 1880; Kulchitsky, 1924a; Garven, 1925; Wilkinson, 1929); 2, methylene blue (Ehrlich, 1886; Dogiel, 1890, 1902; Huber and De Witt, 1897; Wilson, 1911; Hines and Tower, 1928); and 3, reduced silver (Cajal, 1903, 1910, 1925a; Bielschowsky, 1909; Boeke, 1909a; Schultze, 1918; Stöhr, Jr., 1921, 1928; Hinsey, 1927). Both the methylene blue and gold chloride methods offer the advantage of following nerve fibers for considerable distances in their branchings finally to the endings in teased preparations, but they do not give the detail of the fibers and their endings that is to be obtained in silver preparations. The silver method has the advantage of giving excellent neurofibrillar structure at the nerve endings but has the disadvantage of limiting the observations to sections where it is possible to study but a small portion of the distribution of nerve fibers in the muscle. An artifact that must be constantly avoided is the staining of non-nervous structures (particularly connective tissue) by the silver method. Ranson and Davenport (1931) de-

scribe in detail how it is possible to obtain counts of myelinated and unmyelinated fibers in peripheral nerves by the use of the osmic acid and the pyridine silver methods.

Components of motor nerves. Fibers in motor nerves may arise from three sources: 1, the anterior horn cells whose axons pass via the ventral roots; (2), the dorsal root ganglion cells; and (3), postganglionic neurons in the sympathetic chain ganglia. Sherrington (1894) used experimental methods to study these three components and his classic work is still the point of reference today. None of the sympathetic fibers in motor nerves were found to possess a myelin sheath. The sensory fibers ranged in size (in fresh preparations) from 1.5 to 20μ in diameter and made up from one-third to one-half the myelinated fibers in the motor nerves (cat and monkey). The sensory fibers in motor nerves were larger (up to 20μ) than those in cutaneous nerves where the largest sensory fibers were in the range of 16μ. This latter observation was confirmed in the frog by Dunn (1909). The somatic motor fibers varied from less than 4μ to 22μ (cat), the largest fibers in the nerve being somatic motor ones.

Hay (1901) found that the fibers in the nerve to the pale semimembranosus of the rabbit were definitely larger (up to 18μ) than those in the nerve to the red soleus (up to 13.5μ), a difference which has been confirmed by Ri (1931). Although Hay's counts are inadequate because of a failure to count the smaller myelinated fibers, they showed qualitatively that bulk for bulk, the red and pale muscles are supplied with approximately an equivalent number of nerve fibers. He degenerated the somatic motor fibers to these muscles and showed that again, bulk for bulk, the afferent nerve supply was about equivalent in both of them.

Attention was again focussed upon the importance of the sizes of the constituent fibers in peripheral nerves in determining the form of the action potential, by Gasser and Erlanger (1927) and by Erlanger (1927). Erlanger found that the majority of myelinated fibers in the saphenous (cutaneous) nerve fell in the size range of 5 to 13μ, while, in the femoral (motor) nerve, most of them varied from 12 to 17μ.

Although osmic acid preparations are to be desired in studying myelinated fibers, they are of no value in determining unmyelinated fiber content. That a motor nerve contains unmyelinated postganglionic sympathetic fibers has been known for a long time. They were shown clearly in nerves in which the somatic sensory and motor fibers had been degenerated by Kuntz (1927) and Hinsey (1927). Burns (1933) has counted them in similar preparations from the cat and finds 3600–8200

per sq. mm. of nerve cross section in motor nerves as compared with approximately 20,000 per sq. mm. of cross section area in the saphenous and lateral cutaneous nerves in the same preparation. In other words, the cutaneous nerves contained from 2.5 to 5 times as many unmyelinated postganglionic sympathetic fibers as the motor nerves did.

In addition to these unmyelinated postganglionic sympathetic fibers, there are unmyelinated sensory fibers in motor nerves which terminate in the endomysium and the adventitia of blood vessels (Hinsey, 1927). His qualitative observations were confirmed quantitatively by Ranson and Davenport (1931) who found that, after degeneration of the sympathetic fibers, the nerve to the vastus medialis contained 4 unmyelinated fibers for every 10 myelinated ones (cat). Hinsey (1927) failed to find unmyelinated fibers in a motor nerve (cat) containing only somatic motor fibers. The unmyelinated fibers in motor nerves appear to come from two sources, sympathetic postganglionic fibers and sensory ones, the latter being relatively few in number.

In deafferented motor nerves of the cat, Eccles and Sherrington (1930) have shown there are two groups of somatic motor fibers—large and small—in the motor nerves to the hind limb. They showed branching in both the somatic motor and sensory fibers in the trunks before entering the muscle. Similar branchings have been described in the frog by Dunn (1900, 1902 and 1909), Willems (1911) in the rabbit, McClean (1927) in the dog, and Adrian (1925) and Cooper (1929) in the cat. In the case of the somatic motor fibers, this took place several centimeters from the muscle. Eccles and Sherrington (1930) believed that a large majority of the smaller motor fibers (1 to 7μ) make their exit from the spinal cord as such and only a small portion of them are to be attributed to peripheral branchirgs.

It is evident that the somatic motor fibers in motor nerves are myelinated ones varying in diameter from 2 to 22μ. The sensory fibers similarly show a wide range of diameters from unmyelinated axons to myelinated ones of 20μ. The postganglionic sympathetic fibers in the motor nerves are unmyelinated.

Ventral root (somatic motor) component to skeletal muscle. The observations made upon the action potentials of peripheral nerves in mammals have not revealed a potential wave to correspond to the piling up of somatic motor fibers around a peak of 6μ, described by Eccles and Sherrington (1930). This may have its explanation in the fact that these observations (Gasser and Erlanger, 1927; Erlanger, 1927; Erlanger and Gasser, 1930) were not designed to answer this problem. It would

be extremely interesting to record the action potentials in the deafferented and sympathectomized motor nerve of the cat to see if a B or C spike would occur, or better still to stimulate ventral roots and record potentials from corresponding motor nerves. Blair and Erlanger (1933) have emphasized (and rightly so) that there are somatic motor fibers which actually contribute to an extensive stretch of the action potential record and their potentials cannot be considered as contributing solely to any particular wave or waves of the potential. The validity of this contention is amply demonstrated by our knowledge of the histology of the somatic motor fibers in a motor nerve.

The branching of the nerve fibers in a motor nerve involves not only the somatic motor fibers but also the somatic sensory ones (Eccles and Sherrington, 1930). Almost all of the observers concerned with the histology of skeletal muscle have seen the branching of the intramuscular nerve fibers with several motor end-plates receiving branches from one nerve fiber. Reichert (1851) found a ratio of 1 nerve fiber to 20 to 30 muscle fibers in the sterno-cutaneus muscle of the frog. Tergast (1873) made counts of the nerve fibers in a number of motor nerves and compared the total numbers of nerve fibers to the total numbers of muscle fibers counted in cross sections of the muscles. The following are some of his ratios: 1 nerve fiber to 3 muscle fibers in extrinsic eye-muscle of sheep; 1 to 2–3 in human eye-muscle; 1 to 80–125 biceps femoris of dog; 1 to 40–60 sartorius of dog. Mays (1886) observed that the branching of nerve fibers in motor nerves was limited to the portion of the nerve close to the muscle. Dunn (1900, 1902, 1909) presented ample evidence for branchings of both sensory and motor nerve fibers in the motor nerves of the frog. Lucas (1909) found 9 to 10 nerve fibers, which are all motor according to Adrian and Zotterman (1926), in the nerve to the dorsal cutaneous muscle of the frog. With 150 to 200 muscle fibers in this muscle, the innervation ratio would be 1 to 15–20. When counting the nerve fibers in the nerve to the rabbit's masseter muscle, Willems (1911) observed that they increased from 29 to 58 per cent as the more distal sections were counted. This would indicate peripheral branchings, if peripheral anastomoses were ruled out. Stefanelli (1912) illustrates a motor fiber in a cameleon which supplies 35 end-plates.

Adrian (1925) counted the nerve fibers in the main trunk and those in the branches of the trunk to the tenuissimus muscle in the cat. The individual fibers divided in the trunk so as to send branches to both parts of this muscle. Bors (1926) obtained a ratio of 1 to 6 in the extrinsic eye-muscle of man and of 1 to 50 in the semitendinosus muscle.

Fulton (1926, p. 369) has used data from several papers and has calculated the ratio for the tenuissimus muscle of the cat to be 1 to 140. McClean (1927, in nerves to extrinsic ocular muscles of dog) and Cooper (1929, in motor nerve to sartorius muscle of cat) describe branchings of nerve fibers in peripheral nerves.

Clark (1931) carried on the work of Eccles and Sherrington (1930) to determine the number of muscle fibers in a motor unit. By degeneration procedures, he prepared motor nerves in the hind extremity of the cat in which the only myelinated fibers present were somatic motor. He counted the number of nerve fibers in the cross sections of osmic acid preparations of motor nerves and then counted the number of muscle fibers in cross sections of the respective muscles cut at appropriate levels. In the soleus (slow), one motor nerve fiber supplied 120 muscle fibers and in the extensor longus digitorum (rapid), one motor nerve fiber innervated 165 muscle fibers. These are averages, of course. Due to the fact that the small motor fibers to muscle spindles probably branch but little if at all, the average motor unit supplied by the larger motor nerve fibers is probably higher than Clark's averages indicate. The muscle fiber in the soleus weighed 0.122 mgm. (average) and developed a tension of 84 mgm.; in the extensor longus digitorum, each fiber weighed 0.072 mgm. and developed a tension of 48.5 mgm. The tension per each nerve fiber (tetanus) was on the average 9.9 grams in the soleus and 8.6 grams in the extensor longus digitorum. The slow soleus contained larger individual muscle fibers which developed a greater average tension than the smaller fibers in the rapid extensor longus digitorum. The difference in the average motor unit tensions is not great due to the fact that the former motor unit contains on the average fewer muscle fibers than the latter.

Skeletal muscles in mammals are made up of a good many thousand individual muscle fibers. Due to the fact that there is branching of the somatic motor fibers, both while in the nerve trunk and while within the muscle, each anterior horn cell supplies a number of muscle fibers. Sherrington (1929) wrote, "The muscle with its nerve may be thought of as an additive assemblage of motor-units, meaning by motor unit an individual motor nerve-fibre with the bunch of muscle-fibres it activates." Eccles and Sherrington (1930) suggest that the soleus (cat) contains 200 motor units, extensor longus digitorum 330, medial gastrocnemius 450, and tibialis anticus 380. Each motor unit obeys the all-or-none law (Porter and Hart, 1923; Porter, 1929; Sherrington, 1929; Eccles and Sherrington, 1930) and the gradation of response in a muscle depends

upon the relative number of motor units which are brought into play. Sherrington (1931) discusses the significance of the motor unit in the quantitative management of contraction in lowest level coördination.

With the branching taking place in somatic motor fibers in the nerve-trunk as well as within the muscle itself, it is of some importance to consider how the branchings from a single fiber are distributed within a muscle. By counting the nerve fibers in the main nerve trunk and in its branches (nerve to the tenuissimus muscle in the cat), Adrian (1925) was able to prove that the individual fibers in this nerve divide and send branches to both parts of the muscle. The result of this would be that both halves of the muscle would contain muscle fibers belonging to one motor unit and that both halves of the muscle would contract in a "unit" response instead of in a fractional response limited to one-half of the muscle. Such a distribution of motor nerve fibers explains Kühne's well-known gracilis experiment on the gracilis muscle in the frog in which axon-reflexes are demonstrated so well (for description see Starling, 1920, p. 254). Cooper (1929) carried Adrian's work further, for she was able to show that the nerve branches in a motor unit supply a chain of muscle fibers rather than a group of fibers side by side in a parallel-fibered muscle like the sartorius of the cat. A loss of effectiveness would thus be prevented due to the fact that in such a fractional contraction there would be a response in both halves of a muscle and throughout the length of each half instead of in just a localized portion. She also believed that "the tendons and tendinous tissue are so arranged that each fibre has a double connection and thus has a chance to register without loss of effectiveness." It would be of considerable interest to know the distribution of the branchings of the individual motor fibers in a number of different muscles.

Motor endings. After their branchings, the somatic motor fibers eventually terminate in motor endings. These endings may be classified in general into ordinary motor end-plates (terminaisons en placque), grape-like terminations (terminaisons en grappe), ultraterminal endings, and motor endings on intrafusal muscle fibers of muscle spindles. The incidence of these types varies in the different classes of vertebrates.

Doyère (1840) described the terminations of the motor nerve in the muscle fibers of the tardigrades where he saw the nerve coming into relation with the muscle fiber in a little hillock which has been called the "eminence of Doyère." Rouget (1862) saw terminations in reptiles, birds and mammals and they are sometimes called the "terminal nerve-plate of Rouget." Kühne (1862) described the terminal expansion of

the axis cylinder of motor nerves in frog's muscle and in 1864 he gave the first accurate account of the hypolemmal position of the end-branches of the nerve fiber, with a continuity of the neurilemmal sheath with the sarcolemma and the cessation of the myelin sheath at the point of entrance into the ending. Kühne (1887) showed in his 324 figures the great variety of form in the motor end-plates found throughout the vertebrate scale. With the methylene blue method, Huber and De Witt (1897) studied the innervation of muscles in a number of different vertebrates. Grabower (1902), Cajal (1904), Tello (1906b, 1917, 1922), Boeke (1909a, 1911, 1927, 1932), Murray (1924), Iwanaga (1925), Garven (1925), Tiegs (1923a), Hinsey (1927), Wilkinson (1929, 1930a, b), Hines (1931), Tower (1931a) and Heringa (1931) report observations which bear upon the histology of the motor end-plate.

The motor fiber remains myelinated generally till it reaches the motor end-plate (Kühne, 1887; Wilkinson, 1929). It may become unmyelinated in some instances before the termination is reached (Kühne, 1887; Hinsey, 1927; Wilkinson, 1929) or it may give off a terminal collateral branch which may be myelinated or unmyelinated and may go to the same termination as the parent fiber or to another one. Wilkinson (1929) found that in 500 endings in the interosseus muscle of the cat, 90 per cent showed the motor fiber myelinated to within 10 or 15μ of the plate. However, in spite of the fact that all of the somatic motor fibers are myelinated while in the nerve trunk, sections may be found in silver preparations where motor end-plates are supplied with unmyelinated fibers derived ultimately from myelinated ones. Hinsey (1927) has called attention to this fact in explaining some of the mistaken interpretations which have been made in the literature of this problem.

The myelin sheath is lost at the entrance to the motor end-plate, if not before, and the neurilemmal sheath becomes continuous with the sarcolemma. The end-branching generally takes place after the nerve fiber has entered the sarcoplasm of the end-plate although it may take place before. The end-branches terminate hypolemmally in the sarcoplasm which is accumulated in the motor end-plate. In this sarcoplasm, there are nuclei which according to Huber and De Witt (1897) are derived from the muscle nuclei, and the embryological studies of Tello (1917, 1922) support this point of view. In gold chloride and methylene blue preparations, the branches are coarse fronds, the finer nature of which is not disclosed by these methods (Wilkinson, 1929). The sarcoplasm of the sole-plate is clear and unstained by methylene blue, while in gold chloride material it has a fine granular appearance through-

out except close to the fronds. In silver preparations, the neurofibrillar structure within the end-branches is evident. The branches take different configurations, do not anastomose, and terminate in end-rings or end-nets. According to Boeke (1932), they never end in a sharp point tapering out into the sarcoplasm.

Boeke (1911, 1916, 1921, 1926a, b, 1929, 1930a, 1932) has described what he has called the periterminal network. In Bielschowsky stained preparations there is found a reticular differentiation, a network of delicate fibrils, with small meshes and somewhat thickened knobs, which is continuous with the neurofibrillar ramifications in the sarcoplasm. It is possible to follow the fibrils of the network through the sarcoplasm to the myofibrils, where they are said to pass into both ends of the isotropic disc. There has been a long controversy whether or not this network really constitutes the "receptive substance" of Langley as was suggested by Boeke in 1921 or whether it is an artefact. After the motor nerve was sectioned, Boeke (1916) found that it degenerated along with the disappearance of the neurofibrillar structure, and that it regenerated along with the regeneration of the motor endings. He (1933 I and II) observed it in many sensory endings. Wilkinson (1929, 1930a) has again questioned whether or not this network is preformed or is artefactual. In his 1930a paper he says that he has examined the silver preparations of Murray, Agduhr and Bielschowsky and in only Murray's material was this network to be seen. Murray (1924) did not obtain a clear-cut picture like that of Boeke. Boeke (1930a) has answered Wilkinson's criticism and given the literature pertaining to this problem. Tower (1931a) saw this network but could not be sure that it was continuous with the terminal branches of the nerve fiber. Heringa (1931) says that the network terminates in the granules of the isotropic discs. That there is a network present in certain of the silver preparations no one doubts but whether or not this network is continuous with the neurofibrillar structure of the end-plate is controversial (Cajal, 1925b, and Tower, 1931a).

Fulton (1921) suggested that the end-plate nuclei in skeletal muscle might be concerned in some way with the transmission of the nerve impulse. Huber and DeWitt (1897) observed that the muscle nuclei were more numerous in that portion of the muscle fiber receiving the ramifications of the nerve fiber. Tiegs (1923a), Hinsey (1927) and Tower (1931a) have all found an intimate relationship between the end-ramifications of the nerve fiber and the end-plate nuclei. Tower (1931a) says that the motor end-plate "ramifications often seemed to terminate

in relation to one of the nuclei of the sole-plate, encircling it or attaching to a black spot, perhaps the nucleolus." A similar intimate relationship of terminal endings and the nuclei of smooth muscle fibers is described by Boeke (1921) and is referred to for heart muscle by Wiggers (1923). In regard to sole-plate nuclei, Boeke (1932) believes that the close application of these nuclei to the neurofibrillar branches points to a reciprocal action of a trophic or chemical nature between them.

Fulton (1926, p. 200) discusses the evidence for and against receptive substances. He believes that the existence of the receptive substance of Langley is not proved. However, he considers the association of motor nerve endings with the nuclei of the innervated tissue is not merely a chance one. It must be realized that not all the branches of a motor ending in skeletal muscle terminate in relation to sole-plate nuclei.

Tiegs (1922, 1923a, b) has introduced still another conception for the manner of termination of motor endings. He does not consider Krause's membrane as a series of parallel structures situated at right angles to the longitudinal axis of the muscle, but rather as a double helicoid which serves to conduct the nerve impulse from the region of the motor ending to outlying portions of the muscle fiber. He has described terminations of the fibrils of the nerve endings and of minute processes of the sole-plate nuclei which go to form a close connection with Krause's membrane. Wilkinson (1929) has criticized this concept very convincingly and believes that there is no good anatomical basis for the double helicoid structure of Krause's membrane or for a connection of the fibrillar branches of the motor ending with it. Inasmuch as Wilkinson has examined teased and mounted material (selected from a wide range in the vertebrate scale) effectively stained with gold chloride, with methylene blue and with Bielschowsky, it seems that he is in a position to speak authoritatively on this matter. Woollard (1931) likewise doubts it.

It is evident that there is a difference of opinion in regard to the ultimate termination of the nerve fibers in the motor end-plate, i.e., 1, in connection with Krause's membrane; 2, in relation to a periterminal network, or 3, in the sarcoplasm of the sole-plate with some of the terminations intimately related to the sole-plate nuclei. In the author's opinion, the last mode of termination would receive the support of the majority of the investigators in this field at the present time.

The granular appearance of the sarcoplasm is due to very small granules which are found throughout the sarcoplasm of the muscle fiber and to mitochondria which have been selectively stained and described by Noël and Boeke (1925) and by Noël (1925). Noël and Pommé (1932)

have reviewed this work on the cytology of the motor end-plate in normal and pathological material. The mitochondria (some granular but mostly elongated, rod-like chondriocontes) are found in the sarcoplasm of the sole about the sole-plate nuclei as far as this sarcoplasm extends.

In specimens from the cat's tongue fixed and stained for mitochondria, Noël (1926) observed that each motor plate was in part surrounded by a blood capillary which bifurcated on reaching the plate. He believed this to be rather a constant feature of the blood supply to mammalian muscle. Wilkinson (1929) has described what he calls a "confluens capillorum" in the region of the motor endings in lizard and rabbit skeletal muscle. He demonstrated this by combining an injection procedure with gold chloride staining. Boeke (1932), while admitting its occurrence in a number of forms, doubts its constancy or even general appearance. He attributes it to a chance forking of the abundant capillaries in the muscle.

The form of the motor end-plate varies in different classes of vertebrates, even between endings in different muscles in the same individual. There may be a small accumulation of sarcoplasm at the point of termination as in reptiles and mammals. On the other hand, in the amphibians (frog) where the nerve terminal branching spreads for a considerable distance on the muscle fiber, there is no accumulation of the sarcoplasm of the ending. Kühne (1887) has given us some beautiful illustrations of the great variety of form of the endings. Boeke (1921, 1932) described the type of ending in Amphioxus; Murray (1924) in Squalus acanthias; Kulchitsky (1924b) and Lawrentjew (1928a) in frog; Kulchitsky (1924a), Boeke (1930b) and Tiegs (1932a, b) in reptiles; Botezat (1906) in birds; and Wilkinson (1929) in frogs, reptiles and a number of mammals.

Grape-like terminations. There is one type of motor ending in skeletal muscle which has been the subject of much discussion as to the origin of its innervation. Tschiriew (1879), who first described them, named them "terminaisons en grappe." They are supplied by either thinly myelinated or unmyelinated fibers and may be epilemmal (sensory) or hypolemmal (motor). When these terminations are hypolemmal there is little, if any, accumulation of sarcoplasm to form a sole-plate. They are found in amphibians (Bremer, 1882; Kulchitsky, 1924b; Hines, 1927; Lawrentjew, 1928a; Wilkinson, 1929); in reptiles (Tschiriew, 1879; Stefanelli, 1914, 1929; Kulchitsky, 1924a; Boeke, 1927, 1930b; Wilkinson, 1929; Hines, 1932; Tiegs, 1932a, b); in birds (Hunter and Latham, 1925; Stefanelli, 1929) and in the eye muscles of mammals (Retzius, 1892;

Huber, 1899; Crevatin, 1900, 1902; Dogiel, 1906; Woollard, 1927a, b; 1931; Wilkinson, 1929, 1930b; Hines, 1931).

The grape-like termination is not a functional type but rather a morphological one. This accounts for the variety of opinions held in regard to the sensory or motor function of such endings. They have been considered as motor endings either of an immature or mature form by Tschiriew (1879), Thanhoffer (1892), Cipollone (1897), Rossi (1902), Perroncito (1902), Stefanelli (1914, 1929) Kulchitsky (1924a), Garven (1925) and Boeke (1927). Their sensory nature was upheld by Bremer (1883), Giacomini (1898), Huber (1899), Crevatin (1900, 1902), Dogiel (1906), and Wilkinson (1930b). Woollard (1931) and Hines (1931) have found some of these grape-like endings hypolemmal and a great many epilemmal in the eye-muscles of the rabbit.

Kulchitsky (1924a) described cerebrospinal myelinated fibers going to ordinary motor end-plates on thick striated muscle fibers and unmyelinated fibers to terminaisons en grappe on thin muscle fibers. He suggested that the latter endings were epilemmal in his gold chloride preparations and believed they originated from the visceral motor system. This work served as an incentive which set Hunter (1925) to search for evidence supporting cerebrospinal motor innervation for contractile tonus and sympathetic innervation for plastic tonus. With the surgeon Royle, he undertook physiological experiments and operations upon humans which are described in Hunter's posthumous paper (1925) and with Latham (1925) he carried out what seem now to be very superficial anatomical studies. The physiological work is criticized by Forbes (1929) and the anatomical work by Hines (1927), Boeke (1927), and Wilkinson (1929). Boeke (1922, 1927, 1929, 1930b) has worked extensively with reptilian muscle. He has criticized Kulchitsky's (1924a) interpretation and believed that most of the terminaisons en grappe in reptiles are supplied by cerebrospinal motor fibers.

The question narrows down to the point as to whether there is a special group of small muscle fibers in skeletal muscle which is specialized for plastic tonus maintenance and is innervated by sympathetic motor fibers and another group of larger muscle fibers which is specialized for ordinary voluntary movements and is innervated by cerebrospinal motor fibers. This question has been answered very decisively in a negative fashion for the reptiles by the experimental work of Hines (1930, alligator) and Tiegs (1932a, python and lizard; 1932b, lizard). In these experiments the sympathetic innervation was left intact and the somatic fibers degenerated. The terminaisons en grappe disappeared with

the degeneration of somatic motor fibers in Tiegs' experiments and he concluded the terminaisons en grappe in the forms he studied represent a distinct form of motor fiber originating in the spinal cord.

Kulchitsky (1924a) stated that the coarse nerve fibers in Python muscle terminate only in thick muscle fibers and the fine nerve fibers in thin ones. Wilkinson (1929) found terminaisons en grappe on muscle fibers ranging from 10 to 60μ in diameter and terminaisons en plaque on those from 90 to 150μ in the lizard, Tiliqua. Boeke (1930b) has confirmed Kulchitsky's work on the Python. In the lizard Egernia, Tiegs (1932b) did not find this selective innervation; here, coarse and fine fibers were found to terminate on one and the same fiber. Hines (1932) observed that the motor end-plate was the more common terminal upon the agranular, and the hypolemmal or epilemmal grape-like ending upon the granular muscle fibers of the boa constrictor. From the time of their first description, the grape-like endings have been taken to be young or immature forms of the ordinary sole-plate (Tschiriew, 1879; Kühne, 1887; Thanhoffer, 1892; Garven, 1925; Wilkinson, 1929; and Boeke, 1930b). Others have considered them mature forms which are found in fully developed individuals (Stefanelli, 1914, and Tiegs, 1932a, b). Wilkinson (1929), while he considers them immature forms, does so with the reservation that they may never develop during the life of the animal to plate-like endings. He bases his judgment that they are immature forms on the fact that the grape-like endings can be arranged in a series showing increasing resemblance to plate-like terminations, and due to their resemblance to structures found in mammalian embryonic material, in young mammals, and in material from nerve regeneration experiments (see also Boeke, 1930b). Regardless of whether they are mature or immature, the experimental work of Hines (1930) and Tiegs (1932a and b) establishes conclusively that the hypolemmal grape-like terminations in reptiles are supplied by cerebrospinal motor fibers rather than by sympathetic ones as was suggested by Kulchitsky (1924a) and Stefanelli (1929) without experimental proof. The discussion of the origin of the grape-like terminations in the extrinsic eye-muscles will be reserved till later.

Ultraterminal fibers. The term ultraterminal is applied to an unmyelinated branch which arises from the motor end-plate branches inside the plate itself and which goes to form an ending in the same or neighboring fiber. A collateral motor branch is defined as a fine myelinated or unmyelinated branch of the motor fiber before it passes into the end-plate proper. This branching may occur some distance from or in the im-

mediate vicinity of the plate. The ultraterminal endings were seen by Arndt (1873). Bremer (1882) described them in frog muscle and Ruffini (1900) in the muscles of the human hand. They have been seen in reptilian muscle by Perroncito (1901), Sommariva (1901), Rossi (1902) and Tiegs (1932b) but were denied by Kulchitsky (1924a). Tello (1917) found them in younger animals and in embryos but most of them disappeared as development proceeded. They have been described for mammalian muscle by Ruffini (1900), Iwanaga (1925), Garven (1925) Boeke (1927) and Hines (1931). Wilkinson (1929) found them more frequently in frog and salamander muscle, only occasionally in lizard muscles, and failed to see them in a large number of preparations of mammalian muscle. The literature seems to show that ultraterminal endings are more frequent in lower forms and in embryonic and young muscle, but they are seldom found in adult muscles of higher vertebrates; they appear to be more frequent in tongue muscles than in others.

Motor innervation of muscle spindles. Kerschner (1888) and Cajal (1888) independently described the motor innervation of the intrafusal fibers of muscle spindles and gave it a true significance. Onanoff (1890) cut the anterior roots in dogs and observed degeneration of a very small number of nerve fibers in the spindle. When he destroyed the spinal ganglia, a few nerve fibers were left intact in the corresponding spindles. Due to a failure to state the degeneration intervals and to describe the endings in the latter series, it is impossible to evaluate these findings. Ruffini (1898) denies the motor function of the plate-like endings to intrafusal muscle fibers of muscle spindles which he first described in 1892. He assigns to them a sensory function on purely histological grounds.

Sherrington (1894) was unable to establish the existence of motor endings in the intrafusal fibers in the hindlimbs of cats and monkeys although he recognized the possibility of their presence. His work (1894, 1897) led him to believe that the intrafusal fibers were largely independent of a trophic innervation from either sensory or motor nerves to the muscle because there was an absence of obvious degeneration for as long as two years following section of their nerve supply. Horsley (1897, cat and dog) and Batten (1898, cat) came to similar conclusions.

Weiss and Dutil (1896) saw plates on spindle muscle fibers which greatly resembled the typical motor end-plate. Ruffini (1898) and Crevatin (1901) attributed a sensory function to them. On the other hand, Huber and De Witt (1897) and Dogiel (1902) considered them as motor terminations. In reptilian muscle, Cipollone (1898) and Perroncito

(1901 and 1902) showed a myelinated fiber bifurcating to furnish a motor-plate to an ordinary muscle fiber and also one to an intrafusal fiber within a spindle. This observation has been confirmed recently by Boeke (1927 in Tropidonotus natrix) and by Wilkinson (1929) in lizard muscle as well as in the musculus panniculus carnosus of the spiny ant-eater).

Tello (1906a) described the degeneration and regeneration of both sensory and motor endings of the muscle spindle. He (1917, 1922) observed sensory endings in the muscle of a chick embryo of five days while motor endings in the spindles appeared in eighteen day embryos. Sutton (1915) does not speak of the motor terminations in his study of the development of the neuromuscular spindle in the extrinsic eye-muscles of the pig. Cuajunco (1927) did not find them in his study of the development of the spindles in the biceps brachii muscle of the pig; but he (1932) has confirmed their presence on experimental evidence from the flexor digitorum sublimis muscle in the forelimb of the cat.

Kulchitsky (1924a) thought it was probable that the motor innervation of the spindle fibers in the Python was derived from the sympathetic. Hunter (1925) extended this concept to the birds and mammals and believed that the intrafusal fibers of muscle spindles receive only a sympathetic motor innervation hypolemmally. Boeke (1927), in his figure 56, shows an intrafusal muscle fiber in the muscle spindle of a cat, four days after section of the spinal roots proximal to the dorsal root ganglia. A plate-like ending is shown in degeneration while the sensory annulospiral ending is left intact. This would indicate a ventral root origin for the former. By selective degeneration of the sensory and sympathetic components to muscles of the cat, Hinsey (1927), Hines and Tower (1928) and Cuajunco (1932) have demonstrated that when the somatic motor innervation from the ventral roots is the only innervation left, the plate-like endings are still present on intrafusal fibers. This establishes the motor function of these endings.

The nerve fibers supplying these plates are the smallest myelinated fibers going to the spindle; they do not arise as branches of other fibers nor give secondary branches of their own (Ruffini, 1898). They could be traced right into the nerve trunk without showing branching. Hinsey (1927) and Hines and Tower (1928) have described these fibers as small myelinated ones which lose their myelin near their terminations. The endings themselves may vary in size from much smaller to larger than the ordinary motor plate (Ruffini, 1898). They may be situated on the spindle muscle fibers either inside or outside the spindle capsule.

The sarcoplasm is relatively scant at the region of the termination but sole plate nuclei may be in evidence.

They are present in the amphibians (Cajal, 1888), reptiles (Kerschner, 1892; Cipollone, 1898; Perroncito, 1901, 1902; Giacomini, 1897; Huber and De Witt, 1897; Boeke, 1927; Wilkinson, 1929), birds (Cipollone, 1897; Huber and De Witt, 1897; Giacomini, 1897; Tello, 1917), and mammals (Onanoff, 1890; Cipollone, 1898; Huber and De Witt, 1897; Garven, 1925; Boeke, 1927; Hinsey, 1927; Hines and Tower, 1928; Cuajunco, 1932). It is quite evident that, as soon as muscle spindles begin to develop as specialized receptors in the amphibians, the sensory and motor innervation go hand in hand.

Matthews (1933) has isolated four types of receptors (A_1, A_2, B and C) in cat muscle by a physiological analysis involving recording responses from single sensory nerve endings. During supramaximal stimulation and the resulting active contraction of the muscle, the response of the A_2 endings accelerated; when the stimulus was slightly submaximal, it ceased. He concluded that the A_2 endings were the annulo-spiral ones and that only during supramaximal contraction did the intrafusal muscle fibers contract. This would point to a higher threshold for the fibers innervating the plate-like endings of the intrafusal fibers and would indicate that at least a portion of the smaller myelinated fibers which Eccles and Sherrington (1930) found in the somatic motor component might innervate muscle spindles. If we take the data from Eccles and Sherrington (1930, p. 347) for the nerve to the medial head of the gastrocnemius muscle of the cat, we find that there are 431 motor fibers in this nerve of which 140 are 7μ or less in diameter. If we can assume from Sherrington's (1894) work that one-third of the myelinated fibers in a motor nerve are sensory, then there should be 215 sensory fibers in this nerve. If according to Creed et al. (1932), two-thirds of the sensory fibers in a motor nerve are distributed to muscle spindles, there would be 144 sensory fibers distributed to muscle spindles as compared to the 140 motor fibers 7μ or less. It would not seem at all unreasonable to assign a majority of the small myelinated motor fibers to the innervation of the intrafusal muscle fibers.

Hinsey (1927) suggested that the structure of the spindle and its endings would be very well suited for stimulation of the sensory endings by the action potentials of the spindle muscle fibers in active contraction. Although Matthews (1933) presented some evidence which would favor the "action potential" type of stimulation, in general he believed that the stimulation of the A_2 endings was mechanical rather than electrical or

chemical. Matthews' work points to the physiological importance of these somatic motor fibers to muscle spindles which have been neglected in the face of very suggestive anatomical evidence.

Plurisegmental innervation of individual muscle fibers. By far the most complete treatment of plurisegmental innervation of skeletal muscle, with both the anatomical and physiological evidence, is given by Fulton (1926, pp. 177–203). He has presented the older work which establishes the fact that a large majority of skeletal muscles in the adult are plurisegmental with reference to their nerve supply. Sherrington (1892) has shown that the more distal muscles in an appendage show more complete spatial fusion in the distribution of the segmental nerves within the muscle than the more proximal ones do. Thus, while stimulation of any one of the ventral roots supplying the muscles of the foot produces contraction uniformly throughout the muscle, the stimulation of one of the roots supplying the sartorius may produce a contraction which tends to be localized in the upper or lower part of the muscle (monkey). The question which primarily concerns us here is whether or not, particularly in the more distally placed muscles, the individual muscle fibers are plurisegmentally innervated.

Fulton (1926) says that Krause (1869) and Eckhard (1849) were unable to find examples of individual muscle fibers with a double motor end-plate. Kühne (1887) found only in rare instances that long muscle fibers in the frog had more than one motor termination but inasmuch as his work was not experimental, we have no way of telling that, in these rare instances, the endings arose from different segments of the spinal cord. It is well known that motor fibers may bifurcate near their terminations to supply two or even more end-plates (Floresco, 1903; Cavalie, 1902; Boeke, 1911; Garven, 1925; Hines, 1927; Wilkinson, 1929). Where two end-plates exist on the same muscle fiber, experimental evidence would have to be produced to show that the endings were from different segments.

The first attempt to approach this problem experimentally was made by Agduhr (1916). In cats and rabbits, the flexor digitorum muscles are supplied from C7–8 and T1. He first sectioned the 1T at its exit from the intervertebral foramen, 32 hours later the 7C was sectioned, and after 58 hours the animal was killed. The motor fibers arising from T1 had degenerated 90 hours; those from C7, 58 hours, and those from C8 were normal. In silver and gold chloride preparations, Agduhr believed that he could distinguish the endings from these different segments by their stages of degeneration. He thought he could demon-

strate motor end-plates from two successive segments innervating a single muscle fiber. Boeke (1917) criticized this work by stating that the rate of degeneration in different fibers was so variable that, with the degeneration times used, Agduhr's method was unreliable. Later Agduhr (1919a) confirmed these observations in the cat where the 7C (96 hours) and 8C (168 hours), also the 7C (96 hours) and 1T (192 hours), were cut and the interosseous muscles in the fore-foot were examined. He also says plurisegmentally innervated fibers are present in the extensors and flexors in the fore-arm. Wilkinson (1929) examined Agduhr's preparations and was completely unconvinced by them. Invariably, what Agduhr had thought to be two endings on one fiber really represented, according to his interpretation, one ending on each of two different muscle fibers. Wilkinson went to the trouble of reconstructing individual fibers in an attempt at reaching a fair interpretation.

Kulchitsky (1924b) examined the sartorius and gastrocnemius of the frog in methylene blue preparations and saw no muscle fibers in these muscles with two end-plates. Garven (1925) in gold chloride material found two examples of long muscle fibers in the panniculus carnosus muscle of the hedgehog where two long fibers showed two motor end-plates, each separated at opposite ends of the fiber. He did not observe any such instances in the shorter muscles. Hines and Tower (1928) were able to confirm Agduhr's observations in the plurisegmental innervation of extrafusal muscle fibers in the ulnar interossei of the kitten where a normal end-plate might be seen lying close beside a degenerating one.

Lawrentjew (1928b) examined the muscles of the frogs (Rana esculenta and temporaria) with the Bielschowsky technique in degeneration experiments. He observed that the contributing segments supply distinct regions of the frog's gastrocnemius with only a small area of overlap (compare with physiological experiments of de Boer, 1927, and Samojloff and Wassiljewa, 1925). No plurisegmentally innervated muscle fibers were encountered in this muscle. In the sartorius of these same forms, there were distinct regional distributions but the nerve fibers from adjacent cord segments coursed together in the same nerve bundles. His method was not quantitative but he observed that plurisegmentally innervated fibers occurred in the sartorius but seldom.

The most carefully controlled investigation upon plurisegmental innervation appearing in the literature was carried out by Mather (1930) on Rana pipiens. The gastrocnemius and sartorius muscles were examined after section and degeneration of separate roots in a series of

experiments in which methylene blue staining was employed. The gastrocnemius of this form was found to be supplied mainly by the IXth spinal nerve (terminology of Ecker and Wiedersheim, 1896) with only a few fibers to the upper pole from the Xth nerve and to the lower pole from the VIIIth nerve. In 28 different experiments on the gastrocnemius where either the IXth or Xth nerve had been cut, 25 showed no instance of plurisegmental innervation, 2 showed one fiber each with a degenerating and a normal ending and one experiment showed 2 fibers with a degenerating and normal ending. In 34 different experiments on the sartorius muscle, 26 showed no plurisegmentally innervated fibers, 6 had one such fiber, one had 2 and one had 3 bisegmentally innervated fibers. These experiments certainly show that the muscle fibers of the sartorius and gastrocnemius of the Rana pipiens are typically monosegmentally innervated and that plurisegmental innervation of the fibers is extremely rare.

Although Cuajunco (1932) states that he has seen as many as four motor end-plates on a single muscle fiber in the flexor digitorum sublimis and profundus muscles of the cat, this was not Wilkinson's (1929) experience. While he found examples of two immature end-plates on a muscle fiber in the frog and salamander, he has never observed more than one mature ending on a muscle fiber either in the frog, salamander or higher vertebrates. Inasmuch as his observations were made with the gold chloride method, with teasing, they are significant. He examined 2 interosseous muscles (cat) which were teased from end to end and pieces of tissue from different parts of two others. He did not find one doubly-innervated fiber. He estimates that there are about 70,000 muscle fibers in each of these muscles and, if plurisegmental innervation existed in 1 per cent of the cases, it would be present on 700 fibers so that it would seem improbable that he would have missed them all.

Tello (1917) has drawn an analogy between an egg and a muscle fiber by calling attention to the fact that, just as once an egg is fertilized it becomes impervious to the entrance of more spermatozoa, so a muscle fiber once contacted by a growing nerve fiber prevents the formation of any more end-plates. However, he has shown that, in embryonic and young material, a single motor ending may be formed not at the end of a single fiber but by a number of nerve fibers; because (he believes) of the neurotropic action of the muscle fiber upon the nerves in the tissue. Cajal (1925b) has ascribed it to an error of evolution of the terminations. To Boeke (1927, 1932) and to Wilkinson (1929) it has meant something else. Boeke believes that its explanation lies in the method of develop-

ment of efferent nerves. In the tongue, he says, there forms a distinct nerve plexus with an actual fusion of neurofibrillar structures and their protoplasmic envelope. From this "preterminal network" spring the terminal fibers that form the motor endings (Boeke, 1927, pp. 595–596). This network is said to be demonstrable in the tongue with its short muscle fibers but in muscles where the fibers increase in length during the development of their nerve supply, it is not clearly seen. Tello's (1917, 1922) work on the development of motor nerves does not give support to such a plexus with peripheral anastomoses. Boeke (1917) described the formation of a nerve plexus with an outgrowth of terminal branches and endings during the regeneration which follows the section of a motor nerve. If such a plexus formation does take place and exists in the adult stage even after the endings have reached a mature form, it would be conceivable that a single motor ending might receive impulses from more than one segment, or in other words be plurisegmentally innervated through one motor fiber and its end-plate. If in the development, one motor fiber passes out and branches and then these branches pass directly to the muscle fibers, it is inconceivable that there could be any extensive plurisegmental innervation of individual muscle fibers. It is fairly well established that the occurrence of more than one end-plate (from different segments) in a single muscle fiber is a rare phenomenon. On the basis of the evidence at hand, it is difficult to ascertain how much of a plexus formation is present in the adult muscle.

Inasmuch as Fulton (1926) has summarized the physiological evidence bearing on this problem, only the later papers will be briefly mentioned here. A muscle, which is plurisegmentally innervated, is stimulated through separate ventral roots and then the roots are stimulated together. The physiological changes in the muscle have been measured by recording tensions, heat production and action potentials. If two roots a and b are stimulated, the sums of the tensions developed on stimulation of each root alone are added and compared with the tensions developed when both roots are stimulated together. If $Ta + Tb = T(a+b)$, then the evidence favors monosegmental innervation. If $Ta + Tb$ is greater than $T(a+b)$, then there must be plurisegmental innervation, provided all artefacts are ruled out. The results are controversial on this phase of the evidence.

There are some errors involved in some experiments which Fulton (1926) has enumerated, i.e., shortening which occurs during contraction and the diagonal arrangements of the fibers in some of the muscles. Brodman and Cattell (1930) still believe, however, that tension records

give a reliable approximation of the proportion of fibers in a muscle having functionally active nerve endings from the nerve roots. Boyd (1926), Quednau (1926), Weiss (1927), de Boer (1927), Stevens and Karrer (1929), Hintner (1930) and Fischer (1932) give evidence which in general favors monosegmental innervation. Fischer's work (1932) tends to confirm Katz (1925) in the evidence with heat production measurements in frog muscle. However, Cattell and Brodman (1930) believe that myothermic measurements are unreliable when applied to this problem. They showed in 7 preparations that the value of the heat-tension ratio increased more than 50 per cent as the tension increased from a low value to the maximum. They believed that the explanation of this lies in the difficulty in measuring a constant proportion of the heat produced when a changing number of fibers are active.

Beritoff (1927 I and II), Cattell (1926, 1928) and Brodman and Cattell (1930) have presented additional evidence for plurisegmental innervation. Cattell (1928) has reviewed this question again very completely. There is a general agreement that the sum of the action potentials obtained by separate stimulation of the two roots of the gastrocnemius approximates that obtained by their simultaneous stimulation. However, Beritoff (1927 I) has shown that when the lead-off electrodes were placed in the nerve-free portion of the sartorius or the vastus externus, the sum of the action potentials produced when the two roots were stimulated separately was greater than that recorded on simultaneous stimulation of the roots (from 3 to 17 per cent) On the other hand, these two values tended to be equal when the lead was taken from a portion of the muscle rich in nerves. Beritoff believes this supports plurisegmental innervation of the fibers.

In a great many of the experiments where tension has been measured (frog muscle), $Ta + Tb$ has been shown to be greater from 11 per cent (Fulton, 1925) to 70 per cent (Cattell and Stiles, 1924, and Beritoff, 1925). Even in Boyd's (1926) experiments, where great care was taken to prevent shortening of the muscle, it was found that the highest possible estimate of fibers having a double innervation is 23.5 per cent. If we attribute these smaller per cents of overlap to artefact as Fulton (1926) does, we can say that the individual fibers are monosegmentally innervated. Even if we admit that there is a 10 to 20 per cent overlap, we must recognize that it is probably not due to more than one ending on a muscle fiber (Mather, 1930). If it is to be attributed to the persistence of a preterminal plexus, it is astounding that the overlap is so small.

Sensory component to skeletal muscle. The motor nerve passing to

skeletal muscle contains from about one-third to one-half sensory fibers, which vary in size from unmyelinated ones to 20μ in diameter in the cat and monkey (Sherrington, 1894). Ranson and Davenport (1931) showed that in the nerve to the vastus medialis, there are four unmyelinated fibers to every ten myelinated ones in the nerve, which would mean a 1:1 ratio between unmyelinated and myelinated sensory fibers if we assume that two-fifths of the myelinated fibers are sensory. Eccles and Sherrington (1930) confirmed in the cat the observations of Dunn (1909) in the frog, that sensory fibers divide in the trunks and have shown further that the sensory fibers to skeletal muscle divide.

The sensory nerve fibers pass into skeletal muscle and terminate there in two general types of endings, those which are in direct contact with muscle fibers (epilemmal), and those which terminate in the connective tissue of the muscle (interstitial). In the first group there are the grape-like terminations which have been interpreted as sensory by some observers, terminaisons en panier at the extremities of muscle fibers, the epilemmal terminations such as those in the extrinsic eye-muscles, and the sensory terminations in the neuromuscular spindles. Among the second group of interstitial endings, there are terminations which seem to end free and unencapsulated in the connective tissue and those which are encapsulated, like Pacinian corpuscles and tendon spindles.

Huber and De Witt (1897) and Hines (1930) have thoroughly reviewed our knowledge concerning the muscle spindles and their innervation. In the mammal, these structures lie parallel to the direction of the ordinary muscle fibers and are situated generally in the fleshy parts of the muscles. They vary from 0.75 mm. to 10 mm. in length and from 0.08 to 0.25 mm. in width. Each spindle has a lamellated connective tissue sheath which completely surrounds its capsular portion. At its proximal end, the capsule becomes continuous with the connective tissue of the muscle and at its distal end with the perimysium or the tendon. The muscle fibers within the capsule (intrafusal) are surrounded by a connective tissue sheath which Sherrington (1894) called the axial sheath. Between the capsule and the axial sheath is a lymphatic space (Sherrington, 1894) which is filled with fluid and is bridged by connective tissue strands. This space may be from 40 to 60μ in its widest part. Each typical muscle spindle has one or several parent muscle fibers which enter the capsule at its proximal end. Within the capsule each parent fiber divides into two or three daughter spindle or intrafusal muscle fibers which insert into the tendon at the distal end of the capsule or close to it. The portion of the muscle spindle which is surrounded by the

capsule is called the capsular region while the uncovered portion is called the extracapsular region. The latter may be divided into two portions: the muscular extremity, which consists of the bare expanse of parent muscle fibers; and the tendinous extremity, where only a short expanse of bare muscle fibers, if any, is present before the tendinous insertion occurs. The intrafusal muscle fibers have a higher sarcoplasmic content than the ordinary muscle fiber and were thought by Sherrington (1894) to be red fibers. They are circular in cross section, varying from 0.006 to 0.028 mm. in diameter, being from about one-fifth to one-half as thick as the ordinary muscle fibers. They have a central core of sarcoplasm, in which their nuclei are embedded, and a peripheral coarsely striated portion. In the equatorial region, where the afferent nerve endings are found, the nuclei are numerous and the striations are not seen.

The small myelinated somatic motor fibers which supply the plate-like endings in the spindle may approach the spindle with the sensory fibers or may be separated from them. The motor endings may be found either in the proximal or distal polar portion of the intrafusal muscle fibers or they may have an extracapsular position (Ruffini, 1898).

Three to four (and even more to large spindles) sensory nerves pass to the spindle, large myelinated fibers up to 18μ in diameter. These fibers lose their sheath of Henle as they pierce the capsule at different points, but the myelin sheaths are not lost till the nerves reach the axial sheath in spite of the fact that branching may take place before. Ruffini (1898) described two main types of sensory ending, the many variations of which he recognized and which have been seen by other observers: annulo-spiral (primary) and flower-spray (secondary) endings. The annulo-spiral endings, which were supplied by the largest sensory fibers, were arranged as spiral bands around the intrafusal muscle fibers in the equatorial region of the spindle or as a naked axis-cyclinder running lengthwise of the intrafusal muscle fibers and giving rise to ribbon-like rings which encircled the muscle fibers. Each intrafusal fiber of the spindle was as a rule encircled by some form of the annulo-spiral ending. Ruffini (1898) contended that the flower-spray ending was different in morphological character and in the individuality of its nerve fibers. He says that the nerve fibers to the secondary endings are never so large as those to the primary and always pass into the spindle at some distance from the entrance of the fibers to the primary endings. These fibers divide into secondary branches, lose their myelin sheaths and break up into numerous varicose axis cylinders of different forms (resembling a spray of

flowers) in the connective tissue about the intrafusal fibers near the annulo-spiral termination. Huber and De Witt (1897) believed that a great many of the flower-spray endings were the terminal endings of spiral ones or of branches from them, but at times they were branches of terminal non-medullated nerves which did not form spirals.

Although Kerschner (1893) did not find annulo-spiral endings in human muscle, they were seen by Garven (1925) in the muscle spindles of human pectoral muscle. Garven also found the primary and secondary terminations in the muscle of the hedgehog. Hinsey (1927) observed that, in the flexor brevis muscle of the cat, there were spindles in which in addition to the annulo-spiral endings there were secondary flower-spray endings, which in his sectioned material he believed to be supplied by separate myelinated fibers of a caliber smaller than that of those going to the annulo-spiral endings. Creed et al. (1932) still adhere to Ruffini's (1898) description of the secondary ending as coming from a separate sensory fiber. They also describe (p. 163) unmyelinated fibers which are said to enter the spindle with the primary sensory bundle to end epilemmally in the axial sheath of the intrafusal fibers. These are not a constant finding and may represent branches from the myelinated fibers. However, they may be unmyelinated throughout their course.

On the basis of the occurrence of the primary, secondary and plate-like endings, Ruffini (1898) classified muscle spindles in the cat into those with a complex ending (with usually one, sometimes two, primary endings; two secondary endings, and a variable number of plate-like endings), those with an intermediate ending (with one primary, one secondary, and several plate-like endings), and those with a simple ending (a primary ending and a fewer number of plate-like endings). However, the morphology of the spindle innervation varies so that a classification of this kind will not hold when applied to mammalian muscles as a whole.

Huber and De Witt (1897) saw what they interpreted as a vasomotor sympathetic fiber in the capsule of the spindle. Agduhr (1919a) observed thin non-myelinated fibers in the spindle capsule. Cuajunco (1927) found branches from the sensory nerves passing to the spindle which coursed into the capsule; sometimes these were recurrent branches. Some of these nerves have been interpreted as vascular (Hinsey, 1927), but Tower (1932) was convinced that the rich confusion of fibers at the poles of the spindles and in the capsule has not yet been thoroughly analyzed. Possibly some of these fibers in the spindle capsule have a sensory significance other than that concerned with the innervation of the blood vessels.

Hines (1930) has made a thorough search of the literature in regard to the occurrence of muscle spindles in mammalian muscles (see also Regaud and Favre, 1904). They seem to be more numerous in the limb muscles than in those of the trunk, head and neck. They are more numerous in the thoracic muscles than in the abdominal ones. They are frequent in the muscles of the thenar eminence in man (Frankel, 1878) and in the intrinsic muscles of the hand, flexors of the fingers and the quadriceps femoris (Onanoff, 1890). They do not occur in the cremasteric (Cavalie, 1902), the ischiocavernosus and bulbocavernosus (Baum, 1900) muscles. Baum (1900) also failed to find them in the muscles of the external ear, in both bellies of the digastric, laryngopharyngeus and the stylohyoideus. They are lacking in the facial muscles of the rabbit (Cipollone, 1897) and in those of the cat, fetal pig, guinea pig and rat (Smith, 1926). They are present in the external pterygoid and masseter muscles of the rabbit (Cipollone, 1897) and in the masseter muscle of the fetal pig (Cuajunco, 1926).

The muscles of the larynx do not contain them (Sherrington, 1897; Cipollone, 1897; Baum, 1900). Sherrington (1894, 1897, 1898–1899) failed to see them in the eye-muscles, the intrinsic muscles of the tongue and diaphragm. Cilimbaris (1910) did not find them in the extrinsic eye muscles of the horse, pig, dog, cat, fox, rabbit, hare and rat. They were present, however, in the roe, stag, goat, cow, wild boar and sheep. Muscle spindles have been denied for the tongue musculature by Sherrington (1894), Batten (1897), Cipollone (1897) and Baum (1900). They have been seen in these muscles by Franque (1890, man), Pilliet (1890, cat), Forster (1894, man), Boeke (1927, hedgehog in muscles attached to hyoid bone) and Langworthy (1924a, fetal pig, adult cat, dog, opossum and rat). Dogiel (1902) has found them in the muscle of the diaphragm of several mammals.

Hines (1930) concluded by saying, "This review of the distribution of muscle spindles uncovers little organization in their occurrence. Unless, perhaps, those muscles which develop in relation to visceral arches and have not since become attached to bone, do not possess these organs. However, it would seem that the greater are the diversity of use and range of activity to which the muscle is put, the greater the number of muscle spindles found in it." Hines and Tower (1928) made a partial generalization that not only are the numbers of spindles within a given muscle approximately similar, but also that the position where they are found varies but little. There was variation from muscle to muscle (foreleg of kitten) in the amount of encapsulation as well as within the muscle itself. They also emphasized the difficulty in trying to

classify the types of terminations found in various muscles as Ruffini (1898) did. The number of spindles in the limb muscles seems to be great enough to account for almost two-thirds of all the afferent nerve fibers in the respective motor nerves (Creed et al., 1932, p. 160).

Neuromuscular spindles are not present in the fishes. However, they have been shown to be present in at least one tailed-amphibian (Triturus torosus, Mather and Hines, 1934) and in the anurans (von Kölliker, 1889; Cajal, 1888; Franque, 1891; Huber and De Witt, 1897; Dogiel, 1890; Sihler, 1900; Hines, 1930). They have been found in the reptiles (Ranvier, 1878; Perroncito, 1901; Huber and De Witt, 1897; Kulchitsky, 1924a; Boeke, 1922, 1927, 1930; and Hines, 1932), birds (Huber and De Witt, 1897; Giacomini, 1897; Cipollone, 1897), and mammals (Kühne, 1863; Kerschner, 1888; Onanoff, 1890; Sherrington, 1894; Huber and De Witt, 1897; Ruffini, 1892, 1896, 1897, 1898, 1899, 1900, 1901; Dogiel, 1902; Boeke, 1927; Hinsey, 1927, and Hines and Tower, 1928). It should be remembered that there is considerable difference in the structure of the muscle spindles from class to class and the structure as described for the mammalian spindle cannot be applied to spindles from the amphibians, for example. The development of neuromuscular spindles has been described for the fetal pig by Sutton (1915) and Cuajunco (1927) and for birds by Tello (1917, 1922). Huber (1900), Tello (1906a), and Boeke (1916–1917) have dealt with the degeneration and regeneration of the spindle nerve fibers, the work of which is reviewed by Cajal (1928).

Plurisegmental innervation of muscle spindles. In his preparations of the muscle interosseus digiti II from the forelimb of the cat, Agduhr (1919a) claimed to have evidence for a bisegmental sensory innervation of the muscle spindles from C7 and T1. Wilkinson (1929) saw Agduhr's preparations and came to the conclusion, "that the degenerated endings which Agduhr regarded as degenerated sensory endings, were in one case most probably the degenerated motor endings and in the other were a part of the sensory ending, all of which had not degenerated although its fiber supply showed increasing signs of degeneration as it was traced away from the spindle."

While not at all certain about it, Hines and Tower (1928) thought that it is probable that both the motor and sensory terminations upon any particular spindle in the interosseous muscles of the cat are derived from the same spinal segment. They suggest, however, that further investigation is necessary. Hines (1930) was certain that the muscle spindle of the alligator is bisegmentally innervated. Lawrentjew (1928b)

found a spindle in the frog which received sensory fibers from one segment and motor ones from another.

Cuajunco (1932) has repeated Agduhr's work on the flexor digitorum sublimis and profundus muscles of the cat and has used great care in endeavoring to control his experiments. He says that, in Bielschowsky serial stained sections of his degenerated material, he has evidence for mono-, bi- and trisegmentally innervated muscle spindles in these muscles. He doubts that it is to be attributed to chance variation due to the frequency of occurrence. In his cases of plurisegmental innervation of the spindles, the sensory fibers came from consecutive dorsal root ganglia, which is contrary to Agduhr's observations. It is to be regretted that his illustrations are not clearer and that he has not shown a normal ending (as in his figs. 1 and 3) adjacent to a degenerating one rather than just showing the normal fibers. If these plurisegmentally innervated spindles exist as Cuajunco describes them, they would be of considerable physiological interest because stimulation of one receptor may elicit impulses which travel directly to more than one segment of the spinal cord.

Unencapsulated endings in muscle. The muscles of the trunk of fishes form two lateral masses which extend the length of the body. These lateral masses are segmented into myomeres, which are separated by the fibrous connective tissue of the myocommes, on the surface of which the muscle fibers take insertion. There is a similar structural relationship present in the trunk of urodeles, and in a poorly developed state in the adult anurans. In the fibrous tissue of the myocommes there is a rich plexus of myelinated fibers which divide and subdivide and finally give rise to fine, varicose unmyelinated fibrils. These fibrils surround the extremities of the muscle fibers in epilemmal endings which have been called "terminaisons en panier." Each muscle fiber is provided at each end with a "terminaison en panier" in addition to the somatic motor ending which is near the middle of the fiber. These were found by Giacomini (1898) in selachians, teleosts, urodeles and larval anurans, by Ceccherelli (1904) in the dorsal musculature of the adult Bombinator igneus, Rana esculenta and Bufo viridis and by Perroncito (1902) in reptiles. In the urodeles, Giacomini (1898) described one myelinated fiber giving one branch for a termination in a tendon and another one which ended in a "terminaison en panier." In addition to the "terminaison en panier," he observed muscle fibers, either single or in groups surrounded by a plexus or "reticelles" of fine unmyelinated fibers which were branches of myelinated ones and terminated in bulb-like enlargements.

In Giacomini's opinion, these endings, both "en panier" and "reticelles," were sensory terminations which were less differentiated than the muscle- and tendon-spindles found in higher vertebrates. He stated that, when the neuromuscular and tendon spindles reach their definitive constitution, as in adult amphibians, reptiles, birds and mammals, the "terminaisons en panier" are no longer found on the extremities of the muscle fibers.

Throughout the animal scale, there are sensory fibers terminating in muscle, some of which have an epilemmal relationship to the individual muscle fibers and some of which are interstitial in that they branch and seem to end freely in the connective tissue. It is difficult to draw a sharp line of distinction between these two because one and the same fiber may give branches, some of which terminate epilemmally and others interstitially. Reichert (1851), Kölliker (1862, 1889), Odenius (1872), Sachs (1874) and Tschiriew (1879) were of the opinion that there were sensory nerve fibers which terminated not only in muscle spindles but also in the connective tissue of the muscle. Landauer (1892) sectioned ventral roots in the frog, allowed six weeks' degeneration, and found fine fibers intact in the muscle, some of which terminated epilemmally to the muscle fibers. Sherrington (1894) likewise obtained experimental evidence supporting small sensory nerve fibers terminating, after branching, in free fibrils in the cat.

Kulchitsky (1924b) employed methylene blue staining in his study of the innervation of frog muscles. He was able to confirm in normal material Landauer's experimental observations that there are in frog muscle unmyelinated fibers, which are branches of cerebrospinal myelinated sensory fibers terminating in a bush of ramifying threads with leaf-like expansions. Sometimes several terminations were found on one muscle fiber. They were epilemmal and possessed no granular sole-plate. Kulchitsky believed these endings corresponded in structure and function with those Giacomini observed in fishes and amphibians and with the grape-like sensory endings found in the extrinsic eye-muscles by Huber, Crevatin and Dogiel. He also warned against confusing these unmyelinated sensory fibers with the sympathetic fibers connected with blood and lymphatic vessels. These endings were seen in frog muscle by Hines (1926).

In pyridine silver preparations of the vastus medialis of the cat in which the sensory component to the muscle alone was present, Hinsey (1927) was able to show sensory fibers which terminated epilemmally close to muscle fibers and interstitially in the connective tissue. While he could

not trace these fibers to large myelinated ones, he could not deny such an origin on the basis of his sectioned material. Hines and Tower (1928) saw similar endings which resembled a "terminaison en grappe" in the normal and sympathectomized abductor digiti quinti of a kitten.

Sherrington (1897) showed that the sensory nerves to the extrinsic eye muscles of mammals do not course in the fifth but in the third, fourth and sixth cranial nerves. The endings of these fibers are found partly in tendon spindles, which are quite numerous, and partly in muscle spindles which, as was demonstrated by Cilimbaris (1910), are present in some mammalian eye muscles. Retzius (1892), with methylene blue-stained preparations, observed what he called "atypical motor terminations" in the eye muscles of the rabbit. However, Huber (1899) interpreted these endings in the eye muscles of the rabbit as epilemmal in position and sensory in function. Crevatin (1900) saw them in the extrinsic eye muscles of cow, rabbit, ass and man, and in the camel (1902) and Dogiel (1906) in man, ass, horse, cow, dog, and cat. These endings have been under close scrutiny again in the work of Garven (1925, rabbit), Wilkinson (1930b, cat), Hines (1931, rabbit) and Woollard (1931, rabbit). They have been shown to arise from the brain stem, and to pass in the eye-muscle nerves to reach their respective muscles. They are most frequent in the insertion third of the muscle (Garven, 1925, and Hines, 1931). Although the absolute proof is not at hand, Hines says there is no morphological peculiarity that does not allow the old interpretation of somatic afferent function. Wilkinson (1930b), Garven (1925) and Woollard (1931) hold similar views. They are not indebted in any way to the sympathetic system for their origin. These papers will be discussed in more detail later on.

Boeke (1917, 1927) described epilemmal endings of hypoglossal origin in the tongue which he believed subserved proprioceptive function. While Langworthy (1924a) saw muscle spindles in the tongue musculature of some animals, they do not seem to be as highly developed here as in other muscles because other investigators have had difficulty in finding them. A careful search of the facial and laryngeal muscles may reveal these epilemmal grape-like endings.

As far as finally determining from an experimental procedure whether or not the epilemmal grape-like terminations in the eye-muscles are sensory or not seems a hopeless task inasmuch as they course in the muscle nerves and seem to have their cells of origin within the brain stem. However, a comparative morphological study involving extrinsic eye-muscles with muscle spindles and those without might be fruitful in

determining the relative occurrence of the different endings in these two types of muscle.

Endings in the connective tissue of muscle and tendon. Kulchitsky (1924a) observed non-myelinated nerve filaments in gold chloride preparations of Python muscle. He was able to trace some of these to their terminations in connective tissue. A type of sensory ending which is characteristic of other tissues beside muscle is the interstitial bush-like termination, "en buissons." They were briefly mentioned by Kerschner (1888, 1893) but Dogiel (1902) described them in detail in the rectus and transverse muscles of the abdomen in guinea pig, rabbit, monkey, dog and man. In 1903 he found them in intercostal muscle. A myelinated fiber divides into several short myelinated branches, each of which breaks up into a large number of unmyelinated fibers ending in leaf-like enlargements placed between the muscle fibers at their tendinous insertions as well as between the tendon bundles. Apparatuses similar to this are frequently found as the simple epitenal endings in the tendons of the lower vertebrates.

Another sensory organ situated in the tendon is the neurotendinous organ or the tendon spindle, the literature of which is completely presented by Huber and De Witt (1900). Golgi's work (1880) was the first comprehensive treatment of nerve endings in tendons. It extended over the vertebrate series: amphibians (frog), reptiles (lizard), birds (sparrow, finch and swallow) and mammals (rabbit, dog, cat and mouse). The endings in the frog and lizard were not encapsulated as those of birds and mammals and would fall into the classification of simple epitenal endings. Here the sensory fiber loses its myelin and forms branches which approach the tendon bundles and spread over their surface in brush-like figures. Huber and De Witt (1900) examined the tendon endings of amphibians, reptiles, birds and mammals with the methylene blue technique. In the mammals, neurotendinous organs were found in the tendons of practically all the muscles studied. They are situated in the transition zone between muscle and tendon and may exceed 0.8 mm. in length. One end of the spindle is inserted into the tendon near its muscular attachment and the other passes into the tendon proper. They are frequently found in tendon bundles arising from muscle spindles. Each spindle is made up of a number of tendon fasciculi which may vary in shape from fusiform to cylindrical and is surrounded by a capsule made up of several layers of white fibrous connective tissue.

One or more myelinated nerve fibers approach the spindle and sometimes branch before entering. Within the spindle, considerable branch-

ing takes place so that unmyelinated branches are finally formed. These may terminate as free fibrils, as granular enlargements or in brush-like terminations, which are found among the collagenous bundles of the spindles, closely applied to them. There may be anastomoses between the branches. Ruffini (1897) described in the cat what he called a "concomitant fiber," a thin myelinated fiber which rarely accompanied the proper fiber to the spindle. When it arrived at the tendon spindle, it lost its myelin, divided into small branches which terminated in enlargements at the muscle-tendon junction or on the muscle itself. This fiber has been seen to terminate in the spindle capsule.

Hines and Tower (1928) saw some peculiar tendon-like organs situated in the connective tissue between the muscle fibers of the abductor digiti quinti and the lumbricales in the forelimb of the kitten. These resembled some of the tendon organs described by Golgi (1880) and also some endings Dogiel (1902) found in the intermuscular connective tissue of the rectus abdominis of the rabbit. In one instance, they saw a single axis cylinder divide in such a way that one branch terminated in one of these tendon-like endings, while the other terminated in the equatorial ending of an intrafusal muscle fiber. This brings to mind the observations of Kerschner (1888) and of Weiss and Dutil (1896) of myelinated sensory fibers bifurcating and sending one branch to a muscle spindle and the other one to a tendon spindle. Hines and Tower (1928) pointed out, "For such a differentiation to hold a meaning for function, it would be necessary that the same axis cylinder carry two impulses unlike in their frequencies or their amplitude." Frequency variation would seem the better explanation. This brings up another point in regard to the position of the tendon organs in the muscle. Regaud and Favre (1904) first called attention to the fact, which was restated by Hinsey (1927), that anesthetizing the patellar tendon and even cutting it away would not remove all of the tendon organs from activity in the quadriceps femoris due to the fact that tendon spindles are found in the fibrous septa which are present in the muscle.

The corpuscles of Vater-Pacini (lamellated corpuscles) are egg-shaped, white structures which vary from 1 to 4 mm. in length and may be up to 2 mm. thick. There is a capsule which is made up of several dozen superimposed plates of connective tissue which are covered with fibroblasts. The blood vessels and nerves enter at one pole into an inner bulb. There is a single myelinated fiber which loses its myelin and the naked ribbon axis cylinder runs to terminate in a swelling at the other end of the bulb. Along with this myelinated fiber, there is an

unmyelinated one which enters the inner bulb and is said to form a network of its own. It is known as the apparatus of Timofeew (Timofeew, 1896; Sala, 1899; Sokolow, 1899; Dogiel, 1910, and Botezat, 1912). Jurjeva (1927) has presented experimental evidence that these fine fibers are of sympathetic origin. The corpuscles of Golgi-Mazzoni are similar in their structure to the Vater-Pacini type with the exception that they are smaller and have a larger inner bulb. They may have a spherical, egg-like or elongated cylindrical form.

These lamellated corpuscles are found in the muscles of the higher vertebrates, being found frequently in birds and mammals. They are said to be situated more frequently near the tendinous insertions and above and below the muscle aponeuroses. It is only seldom that they are situated in the thickness of the muscle. They have been observed and described in relation to muscle by Golgi (1880), Kerschner (1888), Cattaneo (1888), Mazzoni (1890), Ruffini (1892, 1897), Huber and De Witt (1897) and Horsley (1897). Tello (1917, 1922) gives a description of the development of Pacinian corpuscles and of tendon spindles.

It is evident that muscle is supplied with a number of different types of receptors: simple epilemmal endings, muscle spindles, interstitial endings in connective tissue, tendon spindles and lamellated corpuscles. The receptors seem to have been differentiated in their phylogenetic development from more simple and less complex endings found in the lower forms. Even in some mammalian muscles such as the extrinsic eye muscles, tongue, facial and laryngeal muscles, differentiation into the highly specialized receptors does not seem to have proceeded as far as found in some of the muscles of the extremities. It is evident that it would be hazardous to draw any general conclusions from the morphological findings in any one muscle when there are these variations in the state of differentiation in muscles of the different classes of vertebrates, in different genera and species of the same class, and in different muscles of the same animal.

Proprioceptive fibers in cranial nerves. The origin of the proprioceptive innervation of some of the cranial nerves is still problematical. In contrast to an extraspinal position of the sensory neurons of these fibers characteristic of the spinal nerves, some of the proprioceptive neurons involved in the make-up of cranial nerves are to be found within the brain stem. Sheinin (1930) has discussed the literature bearing upon the distribution of the proprioceptive fibers arising in the mesencephalic nucleus of the trigeminal nerve. It is now generally accepted after the work of Johnston (1905, 1909), May and Horsley (1910), Willems

(1911) and Allen (1918, 1924) that the proprioceptive fibers, passing out to the muscles of mastication in the motor root of the fifth nerve, arise in the mesencephalic nucleus of the fifth nerve. Some of these fibers terminate in muscle spindles.

The origin of the proprioceptive fibers in the 3–4–6 cranial nerves passing to the eye muscles is approaching solution. Although nerve cells have been described along the third and sixth nerves by Nicholson (1924), McClean (1925, 1927) and Clark (1927), they do not seem numerous or constant enough to account for the proprioceptive fibers in them. Wilkinson (1930b) found no ganglion cells along the fourth nerve of the cat. May and Horsley (1910) saw no degenerating fibers in these nerves after section of the fifth nerve. Koch (1916) did not see any connections between the fifth nerve and the eye muscle nerves, but Wilkinson (1930b) showed that the trochlear nerve of the cat sometimes forms anastomoses with the trigeminal nerve. In most cases the trigeminal fibers which entered the trochlear, left it again without participating in the nerve supply of the superior oblique muscle. In just a few instances, one and sometimes two of these fibers terminated in sensory endings in this muscle. McClean (1927) suggested the possibility that the smaller cells in the central nuclei of the eye muscle nerves give rise to proprioceptive fibers. On the basis of chromatolytic experiments, Freeman (1927) concluded that the mesencephalic nucleus sends sensory fibers to the eye muscles by way of their motor nerves. Woollard (1931) sectioned the third nerve close to its exit from the brain stem in one rabbit and after 13 days found chromatolysis in the rostral portion of the mesencephalic nucleus. In failing to rule out a possible damage of some of the muscles receiving their innervation from the motor division of the fifth nerve at the time of operation, he has fallen short of convincing proof. This point has been carefully controlled by Sheinin (1933), who has found following unilateral removal of the orbital contents of the cat that chromatolysis was absent in the motor nucleus of the fifth nerve, but was present in the third and fourth motor nuclei and in the homolateral mesencephalic nucleus.

The facial muscles, while not containing muscle spindles, have sensory endings. Davis (1923) demonstrated that the facial nerve contains sensory fibers which he believed mediated deep-pressure pain as well as proprioceptive sensibility. Carmichael and Woollard (1933) denied that deep pressure pain is mediated by the facial nerve but presented experimental clinical evidence which indicated the mediation of proprioceptive sensibility to facial musculature. Wakeley and Edgeworth (1933)

sectioned the facial and auditory nerves between the medulla and the internal auditory meatus proximal to the geniculate ganglion in one Macacus rhesus. After one month, the monkey was killed. It was found that there were 359 fibers (of all sizes from 2 to 12μ) remaining on the degenerated side. These were taken to be proprioceptive to facial musculature arising in the geniculate ganglion. When compared with the 4782 nerve fibers in the normal facial nerve in the same animal, 359 sensory fibers seems like a very small number as compared with the number seen in extremity motor nerves. Thelander (1924) observed some fibers arising in the mesencephalic nucleus passing caudally and appearing to join the fibers of the facial nerve. He suggested that there might be a connection between these nerves.

The origin of the proprioceptive fibers in the spinal accessory nerve has been discussed by Windle (1931). He has presented experimental evidence based upon chromatolytic methods that the spinal accessory nerve in the cat is not purely motor but is a mixed nerve. Sensory ganglion cells were found along its intra- and extracranial course and, in addition, he believed that some of the sensory fibers arose in the 1C and 2C dorsal root ganglia. Likewise Langworthy (1924a, b) believed that the 2C dorsal root ganglion contributes proprioceptive fibers to the hypoglossal nerve in the cat. Hinsey and Corbin (1934) doubted this contribution from the upper cervical dorsal root ganglia to the spinal accessory and the hypoglossal nerves, on the basis of a failure to find degenerating fibers in Marchi-preparations of these nerves after removal of the upper four cervical dorsal root ganglia and suitable degeneration times in a number of experiments. In an experimental study of the innervation of the muscle spindles of the sternocleidomastoid muscle in the mouse, Willard and Lawrence (1933) have shown that there are medium-sized (afferent) fibers passing from the cervical nerves to the equatorial part of the muscle spindle and fine (efferent) fibers from the spinal accessory to the poles of the spindles. It would seem justified to assume that the cervical dorsal root ganglia supply a portion of the proprioceptive fibers to the sternocleidomastoid and the trapezius by way of the cervical spinal nerves. The problem of the origin of the proprioceptive fibers which Langworthy (1924a) has shown to pass in the hypoglossal nerve is a difficult one to settle. In some forms, some of them probably come from Froriep's ganglion. There is a possibility that some of them have an origin from cells within the medulla.

Lemere (1932), in his study of the innervation of the larynx, found that the nerves supplying the laryngeal muscles passed from the medulla

in the bulbar accessory rootlets and contained large myelinated and fine myelinated or unmyelinated fibers. He believed the smaller fibers are sensory and suggested that there is reason to suspect that they arise in the medulla. He calls attention to the similarity in this innervation and that of the eye muscles.

It is quite evident from this only too brief discussion that the proprioceptive innervation of the musculature supplied by the cranial nerves is still far from settled. The histology of the muscles certainly points to the presence of proprioceptive endings in these muscles; some of them are atypical and simple in type as compared with those in the limb musculature but at the same time definitely sensory in their morphology. The intramedullary positions of some of the neurons of origin are to be contrasted with the extramedullary ones characteristic of the proprioceptive neurons in the spinal nerves in mammals. The intramedullary position of primary sensory neurons is to be considered primitive in the spinal nerves inasmuch as it is only found in the lower forms, fishes and larval amphibians (Hinsey, 1933).

Physiological significance of proprioceptive endings in skeletal muscle. Creed et al. (1932) state, "In the existing state of our knowledge it would evidently be rash to attempt to specify the reflex functions of any of the proprioceptive endings which have been described. Nor is it known which among them subserve 'muscle sense'." Reference to papers bearing upon this subject will illustrate the divergence of opinion. Fulton and Pi Suner (1928) concluded that the muscle spindles are the receptors for the knee-jerk and the stretch reflex and that the tendon spindles are the tension recorders and possibly also give rise to impulses which lead to reflex inhibition of the muscle itself. They interpreted the cessation of action potentials during a tendon jerk in a decerebrate preparation as due to a cessation of the activity in the muscle spindles, which lie in parallel to the rest of the muscle during the contraction of the muscle. Upon the subsequent relaxation, the muscle spindles would be activated again with the renewal of tension upon them and the stream of action potentials would appear again. Hoffmann (1928) has criticized this concept and still holds that the silent period is due to a refractory state within the spinal cord centers.

On the other hand Denny-Brown (1928) stated that this silent period is due to the synchronism of inhibitory impulses which is the sequel of any excitation of the muscle. He regards the muscle spindles as the end organs in which the inhibitory impulses are set up, while the tendon spindles are considered as the organs which appreciate tension whether

passive or active. The latter would be the receptors which would be stimulated in the stretch reflex. His main objection to Fulton and Pi Suner's (1928) explanation was that he found the silent period present in isometric contractions, while it should only occur with shortening of the muscle if it depends upon unloading of the muscle spindles.

Adrian (1926) has devised a capillary electrometer with valve amplification for studying the action potentials and has applied it very thoroughly to a study of the responses of the end-organs (Adrian, 1931, 1932). In the sterno-cutaneous muscle of the frog, Adrian and Zotterman (1926) have examined the responses from single receptors and found that end-organs in muscle respond like other excitable tissue.

Using the oscillographic method which he described in 1928, Matthews (1929; 1931a, b; 1933) has studied the responses from single end-organs. Reasons are given (1929) for thinking that some of the cutaneous impulses in the frog travel in the beta wave (Gasser and Erlanger, 1927) and the proprioceptive ones in the alpha wave. Erlanger (1927) had already suggested this. He (1931b, frog, and 1933, cat) observed the responses in single receptors in muscle and agrees with Fulton and Pi Suner (1928) as to the function of the various end-organs there. With isometric recordings, he demonstrated the silent period in contradistinction to the observations of Denny-Brown (1928). In cat muscle, he isolated four types of receptors: A_1 from flower-spray endings in muscle spindles, A_2 from annulo-spiral terminations, B from tendon spindles and C from connective tissue "fascia." He believed that the A_1 receptors were excitatory for the stretch-reflex and the B type were inhibitory. He was undecided as to the central effect of the impulses from the A_2 endings which accelerated with supramaximal stimulation, ceased with submaximal ones and were activated when somatic motor impulses reached the intrafusal muscle fibers.

Pain in muscle. Evidence has been accumulating which indicates that pain is mediated by small nerve fibers (Gasser and Erlanger, 1929; Ranson, 1931; Heinbecker, Bishop and O'Leary, 1933, 1934). On the basis of Sherrington's (1894) work, 20 per cent of the myelinated sensory fibers in a cat's motor nerve are less than 4μ in diameter. The ratio of unmyelinated to myelinated sensory fibers in a motor nerve of the cat is 1:1 (Ranson and Davenport, 1931). It is evident that there is a sufficient quantity of both small myelinated and unmyelinated sensory fibers in the motor nerve to subserve pain conduction. Hinsey (1927) has shown that there are small myelinated and unmyelinated fibers of dorsal root origin in skeletal muscle of the cat, some of which terminate

near blood vessels and others near muscle fibers. Similar endings were seen by Hines and Tower (1928).

The question of the visceral innervation of skeletal muscle. That skeletal muscle receives a somatic motor innervation from fibers, arising within the brain stem and spinal cord and terminating in motor end-plates, is universally accepted. It has been contended by some that there is in addition an accessory innervation or innervations arising from other sources and serving different functions. From an anatomical standpoint, this problem has been a baffling one, mainly because of the difficulty of determining the origin of unmyelinated fibers in skeletal muscle. They may arise from somatic motor and somatic sensory fibers as well as from the thoracolumbar sympathetic system and the experimental approach with degeneration methods is the only feasible procedure to obtain definite answers to this question.

Innervation according to Boeke. In gold chloride preparations, Grabower (1902) saw unmyelinated fibers entering motor end-plates in addition to the ordinary myelinated somatic motor fibers but he interpreted all of these as collateral motor fibers, branches of the myelinated ones. Likewise Perroncito (1902) agreed with Grabower in thinking that the unmyelinated fibers, which Bremer (1882) and Grabower (1902) had seen coursing in the sheath of Henle along with myelinated fibers, were branches of the myelinated ones. In addition to these, Perroncito described another type of fiber which was unmyelinated as far as he could trace it and it penetrated the motor plate to form a distinct intertwining (reptilian muscle). Similar fibers were seen entering muscle spindles. He first interpreted these unmyelinated fibers as sensory but later (1903) he considered them as sympathetic due to connections with the perivascular nerve plexus. Gemelli (1905) interpreted these fibers as ultraterminal ones, not of sympathetic origin.

In Golgi preparations of bird muscle, Botezat (1906) saw two types of nerve fibers ending in skeletal muscle, myelinated and unmyelinated ones. After Boeke's early publications, Botezat (1910) believed that the unmyelinated fibers he had described in bird muscle were accessory fibers and filled a gap in the phylogenetic occurrence of the accessory fibers which Boeke had described in reptiles and mammals. Boeke (1910–1911) pointed out that it was questionable whether Botezat had clearly shown the independence of the myelinated and unmyelinated fibers he described.

Boeke (1909a) showed a series of drawings taken from the skeletal muscle of human and mole (Talpa) embryos and from adult lacertian

muscle. In these illustrations there are unmyelinated fibers for which he has suggested a sympathetic origin and a possible tonic or trophic function. He (1909b) described these accessory fibers more fully. His more complete work in 1911 presents the form and structure of the motor end-plate in the higher vertebrates and that of the accessory fibers and end-plates. He believed, in addition to the epilemmal sensory endings, there are two kinds of hypolemmal terminations, the ordinary motor end-plate and the accessory endings. The latter were supplied by fine unmyelinated fibers which terminated in end-rings or nets and which he believed were derived from the thoracolumbar sympathetic system. One gains the impression from his figures (Bielschowsky material) in these papers that the accessory endings were present in embryonic and young animals and lacking in adult mammalian muscle.

Boeke (1912) examined Bielschowsky preparations of the superior oblique muscle of the cat 3½ days after section of the trochlear nerve close to the brain stem. He found clumps of unmyelinated fibers terminating in what he took to be hypolemmal accessory endings. He thought that all of the components of the trochlear to this muscle had degenerated (certainly a mistake in the light of the work of Wilkinson, 1930b; Hines, 1931, and Woollard, 1931) and that the only possible origin for these accessory endings was the thoracolumbar sympathetic system.

Boeke (1913) was unable to obtain the Perroncito (1902)-Gemelli (1905) fibers in variously stained preparations of lizard muscle. He enunciates six differential points between the Perroncito-Gemelli fibers and his own accessory fibers. In a study of nerve degeneration and regeneration (1916–1917), he again returned to experiment. On page 17 (1917), the different series on the eye muscles are outlined: a. He argues for the presence of the accessory fibers in normal muscle. b. The accessory fibers remain in the eye muscles 3½ to 4 days after section of the eye muscle nerves near the brain-stem while the motor and sensory nerves are *completely* degenerated. c. Three weeks after section of the eye muscle nerves, most of the accessory fibers had disappeared. He was able to find unmyelinated fibers, which had not degenerated, in the nerve branches between the muscle fibers, but the connection of these fibers to the muscle fibers was not clear. Not a single ending is figured for this degeneration time. d. Two weeks after removal of the superior cervical ganglion, he says the accessory fibers are present but fewer than in sections from series a and b. In this series of experiments, Boeke had two leads which, if intently pursued, would have answered this question, but he left it to the work of others to show the reliability

of these two indications. He outlines two series of experiments on the tongue of the hedgehog: a. Following section of the lingual nerve on one side and a degeneration time which is not stated, he found epilemmal terminations which he believed were proprioceptive corresponding with those in eye muscles. b. Six days after section of the hypoglossal, he saw accessory fibers and endings in skeletal muscle fibers which he believed passed in the lingual nerve and corresponded with those he saw in the eye muscle nerves. One is impressed with the shortness of these fibers in comparison with the length of the accessory fibers shown in the eye muscles in the same plate. These may very well be resistant branches from the somatic motor fibers.

Boeke and Dusser de Barenne (1919) removed the posterior root ganglia and cut the ventral roots of T6, 7, 8 and 9 on the right side in one cat. Thirty days after the operation, the cat was killed and autopsy showed that the posterior and anterior roots of these segments with the corresponding ganglia were cut through. No mention is made of whether or not the ganglia were completely removed and no attempt was made to control the possibility of regeneration to some of the near-by intercostal muscles. The motor and sensory elements in muscle from the seventh intercostal space (Bielschowsky stain) were found completely degenerated, while thin bundles of fine non-medullated fibers appeared to terminate hypolemmally in delicate accessory end-organs. These results were interpreted to mean that the muscles of the trunk have a sympathetic accessory innervation, carrying efferent impulses.

Boeke (1921) described a periterminal network in connection with the endings of the accessory fibers. This was seen to surround both the ordinary motor end-plate branches and the accessory endings in the same sole-plate. His observations (1922) on the Tropidonotus natrix indicated that reptilian muscle is supplied by two varieties of somatic motor fibers, those terminating in ordinary motor end-plates and those in terminaisons en grappe. He thought it very likely, however, that some of the fine grape-like endings were supplied by sympathetic fibers, which are said to occur in groups. He (1926a) replied to criticisms of Rio-Hortega (1925) and of Cajal (1925b) by citing degeneration experiments he himself had made and also those with Dusser de Barenne, and those of Agduhr (1919b, 1920) and Kuntz and Kerper (1924).

A complete review of his entire conception of the innervation of skeletal muscle is given by Boeke (1927). He has reëxamined the material from his previous investigations on eye-muscle innervation and in addition performed experiments with longer and shorter degeneration times.

He confirmed his earlier findings with the exception that he found a larger number of accessory endings of thoracolumbar sympathetic origin. However, the degeneration intervals of the new experiments are not stated and the endings not figured for them. It is to be regretted that all of the illustrations are taken from $3\frac{1}{2}$ days' degeneration material instead of from longer intervals in the eye-muscle experiments and consequently he has failed in his attempt to answer Wilson's (1921) criticisms. He refutes Hunter's (1925) idea that the intrafusal fibers of muscle spindles receive only a sympathetic innervation by showing the presence of a somatic motor innervation to them. However, Boeke is inclined to believe that there is also a sympathetic innervation to the spindle muscle fibers although he does not describe it.

Kulchitsky's (1924a) concept of a special group of small muscle fibers innervated by the sympathetic nervous system was again denied by Boeke (1930b) after observations made upon Python muscle. He enunciated the fact again that terminaisons en grappe in reptilian muscle are not a special system of endings supplied by the sympathetic system but belong rather to the cerebrospinal motor system. Nothing in this paper could be construed to support a sympathetic innervation of skeletal muscle; in fact, there were no contributions from the vascular plexuses to skeletal muscle.

Boeke (1932) has described what he calls a nervous terminal plasmodium or sympathetic ground plexus, which consists of delicate interwoven and anastomosing nerve fibers running in strands or flattened bands with scattered nuclei, forming the ends of the sympathetic plexus. It was in abundance everywhere in the smooth muscle tissues, glands, connective tissue and between skeletal muscle fibers, and in his opinion it constitutes the real efferent sympathetic terminal system. Stöhr (1932a) called it a terminal reticulum in the wall of the stomach and believed that it was of lemmoblastic origin similar to that of the neurilemmal sheath cells. He considered it a transition tissue between connective tissue and nervous tissue. As far as the innervation of the gastrointestinal tract is concerned, it goes back to the work of Lawrentjew (1926) and van Esveld (1928) in which they claimed that the interstitial cells and their branches formed a syncytial plexus, the terminal sympathetic plexus, from which arose the endings to the smooth muscle fibers. On morphological grounds, Johnson and Palmer (1931) denied the nervous nature of this plexus and believed that it was connective tissue and supporting in nature. If such a plexus is present in skeletal muscles and peripheral vessels with interstitial cells, which Woollard (1926) believed

were sheath cells, it would be impossible to degenerate it by removal of the sympathetic chain. The whole question of the nature of the ground plexus—whether it be in the viscera or in skeletal muscle—is a difficult one. One group is bound to claim it is of nervous nature specialized for conduction and another will consider it connective tissue and supporting in nature.

In a series of papers, Boeke (1933) has considered problems of innervation, sensory and somatic and visceral motor. In his III, IV and V studies, he has discussed the problem of the terminal nervous plasmodium in great detail and we are particularly interested in the V study. He states very definitely that in spite of all the criticism that has been directed to his previous work, he is unwavered in his conviction that it all is correct. In the hedgehog, after a unilateral section of both the hypoglossal and lingual nerves and a suturing of the central hypoglossal stump with the peripheral lingual one, two months' degeneration time was allowed. Then the central stump of the lingual nerve was sectioned and 14 days later the animal was killed. The ground plexus was still present in Bielschowsky preparations of the tongue in the connective tissue, about the blood vessels and on the skeletal muscle fibers. The endings he describes in skeletal muscle are said to be similar to his previous accessory fibers but it takes a considerable stretch of imagination to compare the accessory fiber in his figure 16 of his 1933 work with those in figures 23 and 24 of his 1927 work. He says the same sort of plexus is present in the tongue of Python and Varanus.

The network was found in the eye-muscles of a bird and in those of the cat. Again he shows a section of the superior oblique muscle only $3\frac{1}{2}$ days following section of the trochlear nerve. It is interesting to note that he says the eye muscles are innervated by the ground plexus and by the formerly described fibers to skeletal muscle fibers. He found no connection between these innervations. The latter smooth-contoured fibers, which terminate in small ring shaped or reticular endings, have been shown not to exist when longer degeneration times were used by a number of workers (Wilkinson, 1930b; Hines, 1931, and Woollard, 1931). He didn't describe a hypolemmal ending for intercostal muscle of the Python and Tropiodonotus but he believes that the ground plexus has the same relationship here he has described for other muscles.

The author of this review has not seen Boeke's preparations. However, after spending considerable time with a number of Bielschowsky preparations he has in his laboratory (some of them were prepared by Prof. H. J. Wilkinson), he is convinced that it isn't that other people

have not seen the so-called "ground-plexus" but rather that they have been unwilling to call structures of this kind nervous. The Bielschowksy stain is well-known to stain connective tissue and in deeply stained preparations this appears black. Especially where the fibers are small as in reticular connective tissue, networks of this kind may be seen. While willing to admit that it is a matter of opinion and that Boeke has had a great deal of experience with the Bielschowsky stain, the author is still unconvinced of the nervous nature of these structures which have been described. It would be tempting to suggest that they would still be present after complete denervation of the muscle but Boeke's answer to this would be that the ground plexus, being syncytial in nature and arising from interstitial cells, would not degenerate even then. It does not seem, however, that he has brought the accessory fibers which he has described into harmony with his "ground plexus." The fact that he admits, p. 369, "Und so musz ich-schweren Herzens-einstweilen den Schlusz ziehen—," that eye muscles have two types of sympathetic innervation, one by way of the ground plexus, another by way of the formerly described smooth accessory fibers, certainly points to this lack of harmony.

Agduhr (1920) has confirmed Boeke's sympathetic accessory fibers in forelimb muscles of six cats when the somatic nerves were degenerated 5 and 6 days. He degenerated some for 45 days with identical results but he hasn't published any figures for this time. Wilkinson (1929) found somatic sensory endings which had not degenerated in some of his preparations. Likewise Kuntz and Kerper (1924) confirmed Boeke in finding accessory fibers and their endings in dog's intercostal muscle 4 weeks after section of the corresponding somatic nerves and in the muscles of mastication 23 days after section of the mandibular nerve. None of these fibers and endings have been illustrated and it is impossible to comment on them. Kuntz (1927) degenerated the somatic motor supply to the fore- and hindlimbs of dogs and used 3 to 6 weeks' degeneration time. He examined extensor and flexor muscles stained with the pyridine silver method. Unmyelinated fibers of sympathetic origin were seen in the muscular rami of the nerves and the majority of these fibers were seen to lie along the smaller blood vessels, including the capillaries. Some of these fibers were seen to leave the blood vessels and to pass and terminate in relation to muscle. Kuntz says they seemed to be hypolemmal.

Langworthy (1924a) found no accessory fibers in the tongue musculature after section and degeneration of the hypoglossal nerve in the cat.

In the white rat, he (1924b) cut the hypoglossal nerve bilaterally, allowed 2 weeks' degeneration and stained the tongue with methylene blue. He found some dust-like endings on the sarcolemma of the muscle fibers. These fibers were derived from the vascular plexus or from the cutaneous fibers and a sensory function for them was not disproved.

Garven (1925) described four types of accessory fibers in the panniculus carnosus of the hedgehog, but inasmuch as his observations were made on normal material, it could not be said that they prove the dual innervation of skeletal muscle. In his gold chloride preparations, he was unable to find endings of accessory fibers in the muscle spindles of the hedgehog, in the skeletal muscle of frog, lizard, rabbit and human (pectoral). Unmyelinated fibers were present in each case but it was impossible to trace the endings which might as well have been in the blood-vessels as elsewhere.

Hines (1930) described a type of unmyelinated fiber which left the blood vessels and appeared to form a "protoplasmic coalescence" with the longitudinal branching of the end ramifications of the motor endplate itself in the sartorius and pectoralis abdominalis of the frog (Rana pipiens). A similar type of fiber was also seen to penetrate the spindle and seemed to contribute to some of the ramifications of the equatorial endings. When the abdominal sympathetic chain was removed and a week's degeneration allowed, this type of unmyelinated fiber disappeared. This type of fiber had a different ending than Boeke's accessory fibers in that it did not form a separate termination but seemed to fuse with the branchings of the somatic motor fiber.

Innervation according to Kulchitsky. In his study of Python muscle, Kulchitsky (1924a) employed the gold chloride method. He saw unmyelinated fibers ending in termaisons en grappe epilemmally in skeletal muscle. In spite of their epilemmal position, Kulchitsky thought these fibers were motor and that it was quite possible that they belonged to the sympathetic system. Similar fibers and their endings were seen in relation to the intrafusal fibers in muscle spindles. Boeke stated that most of these fibers were hypolemmal in Tropidonotus natrix (1922, 1927) and in Python (1930b).

Later in this same year, Kulchitsky (1924b) used the methylene blue method in studying the nerve terminations in the frog. In addition to the medullated fibers of the cerebrospinal system, there were non-medullated ones which terminated epilemmally in leaf-like expansions similar to the endings Huber (1899) described in the extrinsic eye muscles of the rabbit. He decided these were sensory and was far more doubtful con-

cerning specialized sympathetic endings than he was in his work on reptilian muscle, inasmuch as he did not find sympathetic accessory endings in the frog. In the Python, he considered the epilemmal endings as motor and in frog as sensory. Contrary to his findings in the reptilian muscle, however, he saw epilemmal endings on the same muscle fibers which received ordinary somatic motor fibers.

Kulchitsky's preparations convinced Hunter (1924, 1925) that in skeletal muscle there are two innervations, somatic motor to the larger muscle fibers and sympathetic motor to the smaller ones. Hunter and Latham (1925) applied the gold chloride method to the limb, intercostal, extrinsic and sometimes intrinsic eye muscles of fowls, sea gulls and goats. They found little evidence incompatible with the theory that these striped muscles are divided into alternate groups of muscle fibers served respectively by branches from the somatic and sympathetic nervous systems, the former subserving contractile, the latter plastic tonus. In Hunter's (1925) diagrams of the dual innervation of skeletal muscle, the arc for contractile tonus was subserved on the afferent side by endings in the muscle spindles and on the efferent side by the ordinary myelinated motor fibers terminating in motor end-plates on the larger muscle fibers. The arc for plastic tonus shows on its afferent side epilemmal endings on the thin muscle fibers coming from non-myelinated fibers; on its efferent side the sympathetic non-myelinated fibers terminating hypolemmally in grape-like endings on the thin muscle fibers and on the intrafusal fibers of muscle spindles. Latham (1927), influenced greatly by the then unpublished observations of Wilkinson, had become convinced that their previous position was untenable.

Stefanelli (1912) confirmed Gemelli's (1905) work on the nerve endings in Lacerta muralis and viridis and other reptiles and in birds. At that time he did not accept the idea of sympathetic hypolemmal endings to skeletal muscle. However, in 1929 Stefanelli had changed his mind and decided that his work had shown what Kulchitsky had later published. Boeke (1930b) has discussed this at great length and shows the inconsistencies of Stefanelli's position.

Woollard (1927a, b) was then of the opinion that of all the muscles he had examined, the extrinsic eye muscles were the only ones which received a sympathetic innervation and this was according to the Kulchitsky rather than the Boeke plan. His later observations (1931) showed that, when the experimental approach was used, his previous position in regard to the eye muscles was shown to be incorrect and that they did not receive a sympathetic innervation of any kind.

The Kulchitsky-Hunter conception of muscle innervation has been criticized from an anatomical point of view by Garven (1925), by Boeke (1927, 1930b), by Hines (1927, 1930, 1931, 1932), by Wilkinson (1929) and by Tiegs (1932a, b). The most direct information we have on this point is the experimental work of Hines (1930, alligator) and Tiegs (1932a, Python and 1932b, Egernia). These investigators have been able to degenerate effectively the somatic innervation to the reptilian muscle. Hines degenerated all of the somatic innervation and Tiegs the somatic motor. After this was done, neither of them was able to find sympathetic fibers terminating in relation to skeletal muscle fibers in any way; the same can be said for the intrafusal fibers of the muscle spindle. Here after numerous publications on normal reptilian material, experimental procedures have brought to light the real truth of the matter.

Innervation according to Kuré. Kuré and his co-workers have made numerous contributions (1922a, b, 1925, 1927, 1928, 1930a, b, 1931a, b, c, d, 1933a, b) to the field of muscle innervation and muscle physiology. Kuré's views are summed up in two monographs which he published in 1931 (Kuré, 1931a, b). His work may be summarized by saying that skeletal muscle is innervated on the efferent side in four ways. He says first of all that the cerebrospinal ("pyramidal") motor innervation which goes to the ordinary motor end-plate is without meaning for tonus. He believes the small myelinated fibers which are found in motor nerves are the "extrapyramidal" pathways to skeletal muscle and they have a special end-plate of their own. There are the sympathetic accessory fibers and terminations of Boeke. He is convinced there is a dorsal root parasympathetic innervation. Here the preganglionic fibers have a central origin, pass by way of a synapse to postganglionic cells in the dorsal root ganglia. The latter are thought to give rise to fibers which pass to parasympathetic end-plates in the skeletal muscle fibers. The last three (extrapyramidal, sympathetic and parasympathetic) innervations are said to be concerned in the maintenance of muscle tonus, by far the most elaborate scheme yet presented for a mechanism which the most reliable investigations show to be subserved only by the cerebrospinal innervation to skeletal muscle (see Cobb and Wolff, 1932).

Innervation by cerebrospinal motor pathways. Wilson (1921) criticized the eye muscle experiments of Boeke, pointing out the fact that the degeneration times which the latter had used were too short and that, in Boeke's own work, the evidence from longer degeneration times had been rather inconsistently treated. However, Wilson stated, p. 246, "It is therefore difficult, if not impossible, to avoid the conclusion drawn by

Boeke and supported by Agduhr and others from the results of what would appear to be crucial experiments, that these accessory fibers are sympathetic in origin and hypolemmal in their mode of termination." Later evidence has been inconsistent with this statement.

In his study of the development of motor and sensory endings to muscle, Tello (1917) could not find sympathetic accessory fibers in skeletal muscle. Rio-Hortega (1925) and Cajal (1925b) both doubt their existence. Cajal was unable to demonstrate the existence of sympathetic accessory fibers (adult cat and rabbit.) He found it impossible to distinguish with certainty a fine unmyelinated collateral of a myelinated motor fiber from a purely sympathetic fiber.

Iwanaga (1925) examined the tongue, laryngeal, intercostal and extremity muscles of man with a modified silver method and only twice (in the muscle vocalis) in all of these preparations did he see anything which resembled Boeke's accessory fibers. In the posterior cricoarytenoid, thyreohyoid, and other small muscles, he was unable to find a single accessory fiber. In the eye muscles, he saw only a very few of these endings.

However, all of these examinations upon normal muscle, while of some value, do not give any final answer to this problem. The use of experimental degeneration of the somatic nerves to muscles, if properly controlled, should throw the light of truth on this question. Boeke has often said, and again repeats it in his 1933 paper (V), that one positive result is worth a hundred negative ones. However, a positive result can only be of value if it is found in material that is free from criticism as to the degeneration interval, incomplete removal of all the somatic nerves and the possibility of regenerating fibers having grown into it. A thousand positive results would have no meaning if obtained in improperly controlled material.

Woollard (1927a) observed a variety of normal mammals, mammals suffering from deficiency diseases, cats in which the abdominal sympathetic chain had been removed on one side, and cats in which the ventral roots of the lumbosacral plexus had been sectioned and degenerated. He employed the gold chloride, Boeke's modified Bielschowsky, and the intravitam-methylene-blue methods. Preparations stained with methylene blue gave the clearest pictures and the fibers could be followed for great distances in flat preparations. His results were entirely negative for a double innervation of either the Kulchitsky or Boeke type except in the case of the extrinsic muscles of the eye. In the latter, he distinguished a thick and a thin type of muscle fiber. The thin muscle

fiber was found to be innervated by unmyelinated fibers which Woollard regarded as sympathetic with a Kulchitsky (1924a) type of distribution. These observations were made on normal eye muscles. However, in a later paper (1931), he described some experiments which demonstrate the fallacy of calling these unmyelinated fibers sympathetic in origin. He removed the cervical sympathetic trunk with the superior cervical sympathetic ganglion in 5 rabbits which were allowed to survive for 30 days. Methylene blue and silver stained preparations demonstrated clearly that the unilateral sympathectomy had no effect upon the number, distribution or endings of the finer nerve fibers. In one rabbit, he sectioned the oculomotor nerve close to the brain and allowed 13 days' degeneration. Vital injections of methylene blue demonstrated a perfect forest of fibers with their endings on the normal side but, on the operated side, there was complete degeneration of the ordinary motor fibers and also of the innervation of the muscles of the finer fibers. This, he concluded, showed that the unmyelinated fibers which he had considered sympathetic were ones which travelled with the third nerve and were probably sensory with an origin in the mesencephalic nucleus of the V nerve.

Hinsey (1927) isolated selectively the dorsal root, the ventral root and the sympathetic components to the skeletal muscles of the hind limb in the cat. This was done by degeneration of two of the sources of innervation, leaving the third one intact. He demonstrated in pyridine silver material that there were no hypolemmal endings of dorsal root origin to the ordinary muscle fibers, that the hypolemmal fibers found terminating in the intrafusal muscle fibers were of ventral root origin, and that the sympathetic fibers in skeletal muscle were present in the vascular plexuses and did not pass to terminate hypolemmally in skeletal muscle fibers. He emphasized the importance of securing complete degeneration of the somatic motor and sensory fibers and of being certain that regeneration had not occurred.

Using degeneration procedures similar to those mentioned in the previous paper in application to a study of the muscles in the forelimb of the kitten, Hines and Tower (1928) demonstrated in methylene blue preparations that the sympathetic nervous system fails to take a part in the hypolemmal innervation of the intrafusal fibers of the muscle spindles and that the polar endings were of somatic motor origin. Hines (1930) degenerated the somatic innervation to some of the muscles in the posterior extremity in the alligator by removal of the dorsal root ganglia and section of the ventral roots. After 16 days, her preparations failed

to show a sympathetic innervation either to the muscle spindles or the ordinary muscle fibers. This negates both the Kulchitsky and the Boeke concepts of innervation for reptilian muscles as present in the alligator.

In her study of the innervation of the extrinsic eye muscles of the rabbit, Hines (1931) was able to show conclusively that, following removal of both the superior and inferior cervical sympathetic ganglia, the terminaisons en grappe, the naked endings, accessory terminations and the motor end-plates were undisturbed as well as some nerve fibers distributed around the larger blood vessels. The time allowed for degeneration varied from 6 to 69 days. When the oculomotor nerve was sectioned at its exit from the mid-brain (1 to 15 days' degeneration), only the nerves supplying the blood vessels remained. When the cervical sympathetic chain was removed (29 to 59 days' degeneration) and the III nerve was cut on the same side (3 to 16 days' degeneration), all nerve fibers were degenerated or were in the process of degeneration. It has been demonstrated beyond the shadow of any doubt whatsoever that in the four extrinsic eye muscles supplied by the III nerve, the muscle fibers are innervated *only* by axis cylinders which make their exit from the mid-brain. Although she did not find that the blood vessels were completely denuded after sympathectomy alone, they were devoid of nerve fibers following a combination of sympathectomy and section of the III nerve. Her methods did not show whether these fibers which remained about the larger blood vessels after sympathectomy were afferent or efferent. She emphasized the fact that it is impossible to know whether a fiber along a blood vessel is sympathetic or not unless degeneration procedures are utilized.

Hines was led by her observations to suggest that rather than classifying muscles of the eye as thick and thin, it would be better to classify them as coarsely or finely striated regardless of diameter. She was able to confirm Woollard (1927) in finding that the muscle fiber of greatest diameter is innervated by a somatic motor end-plate, and the smaller ones by terminaisons en grappe, with the exception that the smaller fibers may be innervated by typical motor end-plates and the thicker ones may also receive some variation of the terminaisons en grappe. In addition to the finely and coarsely striated muscle fibers in these muscles, she found a rare type of muscle of minute diameter which was innervated by a single finely myelinated fiber which made contact at one of the distal poles and passed along it toward its equator. In its reaction to methylene blue, it resembled the intrafusal fibers of muscle spindles.

Hines' observations in regard to differences in degeneration rates of fibers of different sizes and of branches of larger fibers are particularly significant in showing the source of some previous mistakes in interpretation of observations in degeneration experiments where the interval was too short.

Tower (1931a) studied the innervation of the muscles of the forelimb in the cat, dog and goat in normal and sympathetically denervated material and some in which each of the three components (dorsal root, ventral root and sympathetic) had been isolated by section and degeneration of the other two. She employed the methylene blue, Bielschowsky and gold chloride methods, the success of which she has defended by giving evidence that the standard of each method was adequate for the problem at hand. "Without exception every ending seen on a skeletal muscle fiber was formed either by a myelinated nerve fiber or by a non-myelinated branch of such, and degenerative section demonstrated the somatic origin of these fibers. Endings of sympathetic nerve fibers or of non-myelinated fibers of independent or untraceable origin were in no instance observed. Muscular and vascular innervations were separately derived from the larger intramuscular nerve trunks and at no point contributed in their peripheral distribution." The sole appearance of the morphology of the "accessory ending" in the course of this work was in a case of regeneration of ventral roots, an observation which had previously been made by Hinsey (1927).

One of the most discussed and debated papers in this field is that of Wilkinson (1929). He undertook a study of the innervation of skeletal muscle at the inspiration of Hunter in the anatomical laboratory at the University of Sidney, Australia. He employed the intravitam methylene blue and gold chloride methods on normal material from frog, salamander, lizard, python, echidna, bandicoot, kangaroo, rabbit, goat, horse, mouse, guinea pig, monkey and man—by far the most representative group of forms undertaken by any investigator. Although surrounded in his own laboratory by an atmosphere which favored the Kulchitsky concept of double innervation of skeletal muscle, he was unable to convince himself that there are any hypolemmal endings in skeletal muscle, aside from those which come from somatic motor fibers. As a Fellow of the Rockefeller Foundation, he visited the laboratories of Boeke, Agduhr, Bielschowsky and Ranson, where work on skeletal muscle innervation had been done and where he had the opportunity of examining the original preparations of a number of investigators. It was the author's privilege to work alongside of him for five months while

he was with Ranson, to coöperate in the preparation of some material and to discuss with him his impressions of the material he had seen. He was thoroughly unconvinced by his observations made upon the original preparations of Boeke and Agduhr and has published his criticisms of them in his 1929 paper. The answer to these criticisms was made by Boeke (1930a, 1931) and Wilkinson replied again in 1931. Wilkinson has seen more of the original preparations bearing upon this field than any other investigator and is in position to speak with authority. He is an excellently trained microscopist. He reports (1929) negative findings with regard to the sympathetic innervation of skeletal muscle and is of the opinion that the sympathetic nerves in skeletal muscle supply only the blood vessels.

While at the Institute of Neurology at Northwestern University, he (1930b) repeated some of Boeke's experiments on eye muscle innervation by sectioning the trochlear nerve close to its exit from the brain stem of the adult cat and allowing 3 to 55 days' degeneration in 21 experiments. His material was stained with Bielschowsky method, in a very effective manner. He found that after 5 days' degeneration, a few motor endings had failed to disintegrate. When he searched, he found no undegenerated fibers entering the muscle so that the indications were that fibers and endings within the muscle do not all degenerate with equal rapidity. The fine proprioceptive endings were more resistant than the somatic motor ones and could resist degeneration up into the sixth day. In 4-day material, these endings were very numerous, and several were found in 5-day material. In Boeke's 3½-day material, it is easy to see how there could be incomplete degeneration of some of the fibers and their endings. Wilkinson's findings in this complete series of experiments offer no support whatsoever for a sympathetic innervation of the muscle fibers of the superior oblique muscle. In a later work (1934) he has carried his observations further, to the intercostal muscles and the interossei and lumbricales of the hind limb of the cat. After degeneration of the somatic nerves, the sympathetic nerves were in relation to the blood vessels but he has not found them terminating in muscle fibers.

Coates and Tiegs (1931) stained muscles of the dog's foot with various silver methods 8½ days after section of the dorsal (proximal to the ganglia) and ventral roots in 6–7L and all of the sacral nerves. They found no evidence for a direct sympathetic supply to skeletal muscle in these preparations which contained the sensory and sympathetic components to the muscles studied. When they degenerated the sympathetic supply to the muscles of the fore limb, the innervation of the muscle fibers was not altered.

The ingenious degeneration methods which Tiegs (1932a, b) has utilized in studying innervation of reptilian muscle have shown that in Python and Egernia, when the somatic motor nerves are degenerated, the sympathetic fibers are found only in the vascular plexuses of skeletal muscle (gold chloride method) and do not have any relation whatsoever to the skeletal muscle fibers. This is a confirmation of Hines' (1930) observations on the alligator.

It is quite clear that there is an impressive body of evidence which has accumulated, by the proper use of the degeneration method and with a variety of staining techniques, which indicates that the only hypolemmal innervation a skeletal muscle fiber has is the somatic motor innervation; at least this is true for reptiles and mammals. It would be a mistake to give negative evidence any undue value unless it can be shown that it rests upon secure experimental procedures as well as adequate staining of the muscles after degeneration. It has been shown beyond any doubt that Boeke's (1927) eye muscle experiments are unconvincing because he didn't use long enough degeneration times. His own observations in 1917 showed this. Furthermore, he has not answered the criticisms which have been directed at his one experiment with intercostal muscles. Now instead, he (1933 V) has brought the sympathetic ground plexus into the problem. Only time will tell its significance but it can be safely said that a great deal more work will have to be done to make it convincing that he is not dealing with non-nervous structures in some cases.

Innervation of the blood vessels of skeletal muscle. The literature pertaining to the innervation of blood vessels in general has been reviewed by Stöhr (1928, 1932b) and in skeletal muscle by Hinsey (1928). The cerebrospinal nerves and thoracolumbar sympathetic system participate in the innervation of blood vessels, in such a manner that the blood vessels receive not only an efferent innervation but also an afferent one. According to Woollard and Phillips (1932), the vasomotor fibers pass to the extremity in the cerebrospinal nerves and have precisely the same distribution as these nerves, i.e., those in the median or ulnar nerves are limited to the same territory innervated by the somatic fibers in them. There may be an extension out on to the peripheral vessels from the plexuses in the splanchnic area but this probably takes place for a short distance only from the subclavian and external iliac arteries. The peripheral course of the vasomotor fibers has been under scrutiny in the past few years and among the recent papers bearing on this subject may be mentioned those of Gelding (1932), Coates (1932), Moore and coworkers (1932, 1933a, b, c) and Burns (1933).

Hinsey (1928) studied the blood vessel innervation in pyridine silver preparations of skeletal muscle from the hindlimb of the cat, in which the three innervation components had been isolated. Thoracolumbar sympathetic fibers were distributed to blood vessels possessing smooth muscle and to a small portion of the capillary bed. Sensory fibers were present in the adventitia of the small arteries and veins, arterioles and venules. While the latter nerve fibers extended to the terminal arterioles, they were not found on the capillaries. Branches of these sensory fibers were observed in the adipose and connective tissues surrounding the blood vessels.

Burns (1933) has counted the postganglionic sympathetic fibers in muscle nerves and, while they are fewer than in cutaneous nerves, still there is a goodly number present.

It has been customary to neglect the nervous control of the blood vessels in skeletal muscle and to attribute changes in their vascular bed to chemical or mechanical factors. Hartman and his co-workers (1928a, b, 1929) studied the changes in the vascular bed directly in the sartorius and tibialis anticus of cats under different conditions. Stimulation of the abdominal sympathetic chain and the motor nerves produced dilatation in the capillaries and constriction at times in the arteries and veins. Blalock (1930) believes that, while the vasomotor nerves are responsible for a small part of the alterations in blood flow produced by stimulation of the nerves to muscles, the major effects are due to the mechanical shortening and lengthening of the muscle fibers. Ni (1931) was able to show that muscle contraction can still increase the local blood supply by opening up more capillaries in a muscle poisoned with mono-iodo-acetic acid. Thus lactic acid is not an indispensable part of the mechanism, but that it may produce vasodilatation was shown by Keller, Loeser and Rein (1930). They have made an extensive study of the blood flow in skeletal muscle and they find that the increase of blood flow in muscle during work is due to changes in two parts of the vascular bed, one of which is regulated by vasomotor nerves (precapillary portion) and one of which is independent of nervous influence (the capillaries). Rein and Schneider (1930) have continued this work and have studied the effects of changes in body temperature and blood pressure and of adrenalin upon the blood flow through skeletal muscle at rest and at work. That stimulation of the abdominal sympathetic can reduce the total volume (plethysmograph) of a muscle at work was shown by Baetjer (1930). Bischoff and Ricker (1932) present evidence for a central nervous system control of the blood flow in the abdominal mus-

cles of the white rat. Regardless of what the most important mechanism for the control of the blood supply of skeletal muscle may be, its blood vessels are supplied with nerves, sensory and motor. In this regard the muscles of the head seem to have an innervation from two sources as was shown so clearly in the work of Hines (1931) in the eye-muscles of the rabbit. In addition to the vasomotor fibers from the thoracolumbar sympathetic, there was a contribution to the vascular innervation from the III cranial nerve. It would seem quite likely that the latter fibers would be sensory, although her method did not furnish definite information concerning their function.

Slow and rapid muscles. Since the investigations of Ranvier (1873, 1874a, b, 1889), we have classified muscles as red and pale ones in birds and mammals. Ranvier demonstrated that red muscles contracted more slowly and developed tetanus at lower rates of stimulation than pale muscles. Red muscles exhibited histological differences from pale ones, consisting of a more granular sarcoplasm, a more distinct longitudinal striation, and centrally as well as peripherally placed nuclei (for recent work see Cameron, 1929). These granular fibers with marked longitudinal striations have been found in many species and are not always associated with red pigmentation. The granular and clear fibers occur side by side in the muscles of mammalian forms (Roberts, 1916) and have been homologized with the red and pale fibers in the rabbit. Granular and non-granular muscle fibers occur in certain of the reptiles and amphibians (Hines, 1927, 1932). Grützner (1887) believed that the tonic functions of muscle were subserved by the granular fibers and clonic ones by the clear fibers. Since Hunter (1925, p. 13) suggested, "that the skeletal muscle of vertebrates consists of two sets of muscle fibres disposed in groups, each with its own specific innervation, and consequently, its own specific function," attention has again been fixed upon the histological variations in skeletal muscle. This has been discussed thoroughly by Cobb (1925), Needham (1926) and Hines (1927) and no attempt will be made to review this very extensive literature.

Denny-Brown (1929b) has shown that there is no absolute histological criterion of the speed of contraction of mammalian muscle. His experiments showed that the duration of a twitch, the opacity (granulation) and size of the fiber may vary independently. In the cat (and the same is true for the dog and monkey), there are two groups of muscles which still retain differences in the speed of contraction, in spite of the fact that differences in color are very small in contrast to those in the rabbit. All slowly contracting fibers were found to be able to store lipoid in the

form of liposomes with a resultant granular appearance, while few rapid fibers had this property. Histological differences were found to disappear on emaciation but the differences in the speed of contraction remained. Furthermore, speed differences in the muscle groups of the cat appeared at an earlier stage of growth than the redness of the muscle. Creed et al. (1932) point out that the chief extensor muscles of all mammals have a deep, slow component and a superficial rapid component, i.e., soleus (slow) and gastrocnemius (rapid) in the ankle extensors, vastus intermedius (slow) and vastus lateralis (rapid) in the knee extensors. On the other hand, the flexor muscles are usually the rapidly contracting variety. They present (p. 58) a rather complete table listing the essential characteristics of slow and rapid muscle. Denny-Brown (1929a) has shown that the slow muscles are those in which the stretch reflex is most easily evoked and in which there is a readier response to labyrinthine and neck reflexes with a shorter latent period and a higher tension than is found in the rapid muscles. However, this difference is only relative as both types of muscle are potentially involved in all reflexes.

If there is any fundamental difference between the innervation of the slow and rapid muscles, it should be demonstrable on examination of the motor nerves and their endings. Hay (1901) showed that the motor nerves to the (slow) soleus in the rabbit contained nerve fibers that were smaller (up to 13.5μ) than those to the (rapid) semimembranosus (up to 18μ). Upon degeneration of the somatic motor fibers, there was not a great deal of difference in the diameters of the afferent fibers in the two nerves. He believed that bulk for bulk both the afferent and efferent supply to the two muscles were about equal. He found no essential differences between the motor end-plates and muscle spindles in the two muscles. Ri (1931) degenerated the somatic sensory fibers in both the brachial and lumbosacral plexuses in the cat and found a higher percentage of fibers below 7μ in the slow muscles than in the rapid ones. Cameron (1929) reports that muscle spindles are more frequent in the red muscle than in the pale muscle of the rabbit.

The only essential difference which Eccles and Sherrington (1930) showed between somatic motor fibers to the (slow) soleus and to the (rapid) lateral head of the gastrocnemius was that the peak of the larger fibers in the former fell at about 10μ and in the latter at about 15μ. This difference in size of the larger fibers in the two muscles may be partially accounted for by the work of Clark (1931), who showed that in the cat on the average 1 motor fiber in the (slow) soleus supplied on the average

125 muscle fibers, while in the (rapid) extensor longus digitorum 1 motor fiber supplied 165 muscle fibers. He suggests that in the gastrocnemius the ratio may even be as high as 1:300 or 1:400. It would not be surprising at all that the individual motor fibers should be larger if they supply a greater number of muscle fibers in their units.

While the somatic motor fibers to the slow muscles may be smaller than those to rapid ones, there is no evidence (that will withstand critical examination) to support any fundamental difference in the source of the innervating fibers to these two types of muscles. Garven (1925) found it impossible to divide the muscle fibers of the panniculus carnosus of the hedgehog into definite classes as was done by Grützner (1887). Likewise it was impossible to put the motor end-plates into two definite types, in spite of the fact that they showed variation in size, form and shape. He described all muscle fibers, whether rich or poor in sarcoplasm, as being innervated in exactly the same way.

Significance of trophic innervation. Kuré (1931a) states, after reviewing the work from his laboratory, that it is firmly established that the "spinal-parasympathetic" fibers supply a trophic innervation to skeletal muscle. First of all, Hinsey (1927) and Hines and Tower (1928) have shown that there are no hypolemmal endings of dorsal root origin in skeletal muscle. Furthermore Tower (1931b) and Hinsey (1931, 1934) have presented recent anatomical evidence against the presence of the "spinal-parasympathetic" system which Kuré (1931a) describes. Hinsey (1933) has reviewed the literature showing that there is no conclusive evidence of an intraspinal origin for efferent fibers passing out over the dorsal roots of mammals. The paper of Kahr and Sheehan (1933) has appeared since then in which they have confirmed Kuré in his work on the spinal parasympathetics. Their figures leave some doubt, i.e., figure 1b shows fibers with beaded swellings resembling those in figures 14–15–16 of Hinsey's (1934) paper which he demonstrated to be regenerating fibers following the same procedure with 27 days' degeneration instead of the 69 which Kahr and Sheehan used. Furthermore, their figure 5, illustrating myelin sheath degeneration in a Marchi preparation of a small motor bundle to muscle following intradural section of dorsal roots, has certainly been misinterpreted. Either they have traumatized the dorsal root ganglia or the ventral roots in their experimental procedures.

Tower (1931b) removed the posterior root ganglion supply of the muscles of one forelimb in four cats. In one of them, the stellate ganglion on the corresponding side was also extirpated. The animals

were killed after 4, 6, and 12 months. During life no atrophic or dystrophic changes were noted in the forelimbs which had been deafferented. The corresponding interosseous muscles were weighed and no differences were observed. Microscopic preparations failed to reveal any differences. Diameter measurements of muscles (400 fibers on each side) from the normal and operated side averaged the same. The conclusion was reached that the posterior roots play no essential rôle either alone or in association with the sympathetic innervation in maintaining the normal trophic state of skeletal muscle.

Hunter (1925) described an increase in connective tissue and in the number of small muscle fibers in muscles in the goat's hind limb after sympathectomy. Kerper (1928) reported a general reduction of the diameter of the muscle fibers and length of sarcomeres and the proliferation of nuclei. Gaissinsky and Lewantowsky (1929) found atrophy with loss of longitudinal and cross striations and hyalinization in muscles which had been sympathectomized for four months. Kuré and his co-workers (1925) described a condition resembling progressive muscular dystrophy in one dog, sympathectomized for 10 months, but it was absent in three others which had been sympathectomized for 3, 6 and 15 months. They (1926) observed atrophic changes in the muscles of the face, neck, arms and back following a cervical sympathectomy in man.

Contrasted with these positive findings are the negative ones of Jonnesco and Floresco (1902) in man, and Floresco (1903), Takahashi (1922), Feldberg (1926), Tiegs and Coates (1928), Coates and Tiegs (1930), Nevin (1930), and McCullogh, McFadden and Milroy (1930) in experimental animals following sympathectomies. Tower (1931c) sympathectomized the forelimbs of cats, dogs and goats and permitted the animals to live from one month to two years. In Bielschowsky preparations from the normal and operated forelimbs, she reports: 1, staining reactions of equal intensity in operated and normal material; 2, longitudinal and cross striations equally sharply stained on both sides and no tendency to fracture along either of these planes; 3, no increase in size or number of nuclei, and 4, no increase in connective tissue. Measurements of diameters showed that 2600 muscle fibers from the sympathetically denervated muscle had an exactly equal average diameter to that of the same number of fibers from the normal muscle. She concluded that the local sympathetic innervation exerts no trophic control on the form of the extrafusal muscle fiber in skeletal muscle.

Tower (1932) found that sympathectomy had no effect on the intra-

fusal fibers of the muscle spindles or on the capsule of the spindle of the cat. She studied the muscle spindles of the fourth and fifth interosseous muscles in the forelimb 5, 6, or 12 months after the various lesions. When the ventral roots were sectioned, there was atrophy of the polar portions of the spindle and the capsule thickened. This atrophy and degeneration of the intrafusal fibers was quite in proportion to that of the extrafusal ones and of the same nature. Following removal of the dorsal root ganglia (C6 to T2 inclusive), there was a slight change in the capsule, consisting of a slight increase in diameter and thickness in the region where the bulk of the sensory fibers and endings had been removed by degeneration but there was no general thickening. The polar regions of the intrafusal fibers were unaffected. However, in the equatorial region at the point of termination of the sensory fibers, there was a change, not very noticeable after 4 months, but quite clear after 6 months and a year. It consisted of gradual obliteration of the muscle nuclei in the equatorial region and a conversion of this portion of the intrafusal fiber to a structure resembling the polar regions. She describes apparent hypolemmal endings of the annulospiral fibers in the sarcoplasm but admits the possibility that these may have been artefacts. However, if there is a periterminal network at the sensory terminations with an intracytoplasmic position (for literature see Boeke, 1932), it may be possible to account for these changes at the equatorial region following removal of the dorsal root ganglia.

Cuajunco (1932) found that removal of the dorsal root ganglia caused a preliminary swelling of the spindle as a whole with thickening of the capsule and swelling of the intrafusal muscle fibers with disappearance of the transverse striations. This change took place in an intrafusal fiber for which he could demonstrate no motor endings. In an intrafusal muscle fiber with a motor ending, the appearance was not absolutely normal so that he concluded that the presence of the somatic motor innervation retards pathological changes in these intrafusal muscle fibers following posterior root denervation. The observations of Tower (1932) show that the spindle muscle fibers are dependent upon a trophic innervation from somatic motor fibers in spite of negative evidence from the older work which she quotes. The change which she describes for the equatorial region following removal of the dorsal root ganglia and degeneration of sensory fibers to the spindle is a difficult one to explain. Possibly observations upon the spindle muscle fibers following section of the dorsal roots proximal to the ganglia will throw some light upon this.

Recent physiological evidence bearing upon the question of sympathetic

innervation of skeletal muscle. After having reviewed the evidence bearing on a possible function of the sympathetic nervous system in the innervation of muscle tonus, Forbes (1929) concluded that there was no foundation for the view that the sympathetic system controls a plastic element (Hunter, 1924; Royle, 1924) in tonus functionally distinct from the so-called contractile tonus. Sympathetic nerves on stimulation do not produce a contraction of muscle and, when they are severed, tonus does not disappear. He was of the opinion that it was doubtful that the sympathetic innervation has any appreciable effect on tonus. Bremer (1932) has written an excellent review on the investigations bearing on the problems of muscle tonus and a very complete bibliography is included.

Popa and Popa (1928) and Van Dijk (1930a) have confirmed Hunter's (1924, 1925) observations on the pigeon in showing a drooping and loss of postural contraction in the sympathectomized wing. Royle (1929) has added further observations favoring the maintenance of plastic tonus via sympathetic pathways. McCullogh, McFadden and Milroy (1930) observed an appreciable loss in tonus in the quadriceps femoris following post-ganglionic sympathectomy to the hind limbs of dogs. Outside of this they found no disturbances in the limb muscles either in the resting or actively exercised states and the chronaxie of these muscles was not appreciably changed. Phillips (1931, 1932) removed the abdominal sympathetic chain on one side in 35 cats and examined 23 of them 10 to 80 days later. He found changes in the proprioceptive reflex activity which were progressive and were most permanent weeks after the sympathectomy. He concluded that in postural contractions sympathetic nerves had no direct motor influence on skeletal muscle fibers and that the progressively accumulative changes which followed sympathectomy depended on some trophic alteration modifying the responses of proprioceptive endings. This trophic alteration seemed to result in a concentration at low threshold of the activity of the proprioceptive receptors responsible for the excitation of myotatic contraction. However, in this relation it should be mentioned that Denny-Brown (1929a) found that sympathectomy failed to produce any qualitative changes in the stretch reflex of a muscle.

Kiyohara (1927) and later Hintze and Seager (1929) have obtained no support for the cervical sympathetic pathways having any direct influence on the tonus of ear muscles. Mortenson, Friedbacher and Quade (1928), Tiegs and Coates (1928), Coates and Tiegs (1928), and Tower and Hines (1929) have not found any evidence for a sympathetic innervation

for postural contraction in the goat, having failed completely to support the work of Hunter (1924) and Royle (1924). These experiments answer the criticism that previous workers who reported negative evidence had chosen another experimental animal than the goat. Coates and Tiegs (1928) report that sympathectomized muscle (goat) fatigues more easily than normal muscle in the opposite limb but there were no tonus differences described.

Coates and Tiegs (1928) were able to show that when the sympathetic ganglia, supplying postganglionic fibers to the brachial plexus of the pigeon, were removed so as not to injure the adjacent spinal nerves, no postural defects occurred. They attribute the positive findings of Hunter (1925) and others as due to injury and trauma to the spinal nerves at the time of sympathectomy. Tiegs (1931) showed that when the dorsal roots of the nerves supplying the brachial plexus in the pigeon were sectioned in addition to sympathectomy, no drooping occurred. Bisgard (1931, dogs and goats) and Kuschinsky (1932, rabbits) present additional evidence that the sympathetic nervous system plays no part in the maintenance of postural contraction (tonus).

These observations, which fail to show a direct participation of sympathetic nerve impulses in the maintenance of postural contraction, should not be taken to mean that these impulses are without effect upon the activity of the muscles. The work of Orbeli (1923, 1924) and his student, Ginetzinsky (1922) and others (see Gantt, 1924, 1927; Krestovnikow, 1927; Ginetzinsky and Orbeli, 1927; Ginetzinsky, Nechorosev and Tetjaeva, 1927; Orbeli and Tonkich, 1927; Orbeli and Fiedelholz, 1927) shows that stimulation of the sympathetic retards the appearance of fatigue and influences the functional activity of skeletal muscle. Fulton (1926, pp. 409–413) describes some of this evidence in detail. Orbeli is of the opinion that sympathetic impulses exert an influence upon skeletal muscle much as they do in heart muscle, increasing the strength, rapidity and height of contraction.

Nakanishi (1927, 1928, 1930, 1931a, b, 1932) believes that he has presented evidence in the frog showing that sympathetic impulses exert a regulatory (facilitory or inhibitory) effect upon muscle contraction. Schneider (1929) was unable to confirm Nakanishi and attributes the latter's findings to an escape of current at the point of stimulation. In his fourth paper, Nakanishi (1931a) admits the validity of Schneider's criticism. By an improved method, he thinks that he still is able to demonstrate a facilitory effect in muscle tetanus due to a difference in the configuration of the tetanus contraction curve obtained by stimula-

tion of the ventral roots and that obtained by direct stimulation of the sciatic nerve. He obtained no constant result when he stimulated the abdominal sympathetic trunk simultaneously with the ventral roots.

Wastl (1925) repeated the experiments of Ginetzinsky (1922) on the frog and tried similar ones in the cat with no evidence for any sympathetic effect on skeletal muscle except by modifying the circulation. Fulton (1926) criticized her work by saying that the isotonic lever which she used was poorly designed to bring out small differences in force of contraction. Furthermore, there is evidence from her work that the effect was present and misinterpreted as due to a spread of current.

Baetjer (1930) confirmed in the cat the findings of the Orbeli school for warm-blooded animals (Ginetzinsky, Nechorosev and Tetjaeva, 1927). Her excellently controlled observations showed that stimulation of the sympathetic chain could either decrease or increase the height of contractions in the cat's tibialis anticus induced by a series of single induction shocks applied to the anterior spinal roots. The decrease in contraction heights was shown to be due to vasoconstriction. The increase in contraction heights was not due to an increase in blood supply as was shown by concomitant plethysmographic tracings of the contracting muscle. By cross circulation experiments, she showed that the increased contraction was not due to any substance carried in the blood stream from the splanchnic area such as epinephrin. It was present following injection of ergotoxin which paralyzed the vasoconstrictors. She concluded that the increased contraction following concomitant sympathetic stimulation was due to conduction over fibers arising in the sympathetic chain other than vasoconstrictors and that the impulses affected the muscle in some unknown manner. There are two observations presented in her work which are exceedingly interesting in the light of the later work of Cannon and Bacq (1931) and Cannon and Rosenblueth (1933). First of all she obtained a pressor response in the donor animal in a cross circulation experiment when the lower abdominal sympathetic chain of the recipient animal was stimulated. Secondly, she described an increase in the contraction height of contractions in the tibialis anticus, in which the sciatic nerve had been sectioned, on stimulation of either the ipsilateral or contralateral sympathetic chain. These results could be readily explained on the basis of the elaboration of sympathin (Cannon and Bacq, 1931).

Van Dijk (1930b) has confirmed Orbeli's observations for the pigeon in showing that impulses over sympathetic fibers increase the contraction height of fatigued muscle. In two cases in man following resection of

the cervical sympathetic trunk, Altenburger (1931) reports that the facial muscle on the sympathectomized side fatigued more readily than on the normal side on tetanization of the facial nerve. However, in this relation, it is important to note that Tower (1926), after unilateral removal of the stellate ganglion in dogs, found 5 to 7 months later that the capacity for prolonged muscular work and the onset and severity of the fatigue were not affected by sympathectomy. She tested them, following training, in a treadmill. Campos, Cannon, Lundin and Walker (1929) report similar findings in the dog.

Schilf and Sternberg (1932) describe a decrease in the height of contraction in a cat's tongue (produced by rhythmic stimulation of the hypoglossal nerve) when the sympathetic trunk is stimulated in the neck. They attributed this to a vasomotor effect because it disappeared on ligation of the carotid artery. Stimulation of the chorda tympani was without effect on the contraction of the tongue muscle.

Hartman and his co-workers (1928a) studied the effect of stimulation of the abdominal sympathetic chain on the capillaries and skeletal muscle fibers of the sartorius and tibialis anticus of the cat. On stimulation of the sympathetic, the capillaries dilated, the striations usually became more distinct and there was a peculiar transverse vibration of the fibers which later developed into a transverse twitching. It is extremely interesting that they found that both the vascular and the muscle fiber reactions to sympathetic stimulation resembled the responses to epinephrin.

Britton (1930) and Herrin and Meek (1931) both report a lower glycogen content in sympathectomized (chronic) muscle than in the corresponding muscle from the opposite normal limb. Herrin and Meek find this decreased glycogen content more marked in the postural muscles (quadriceps in dog and hamstrings in rabbit), an observation which is difficult to explain. Furthermore, stimulation of the sympathetic chain tended to produce a loss of glycogen in normal muscle. They state that it seemed that normal sympathetic impulses possess a glycogenetic effect while those produced artificially were glycogenolytic. They suggest the apparent contradiction here could be explained by the assumption of two sets of fibers in the nerves.

The Lapicques (1930) found that, after they had destroyed the spinal cord and arrested circulation in the frog (Rana fusca), stimulation of the sympathetic reduced the chronaxie of both normal and curarized muscle. Altenburger and Kroll (1932) describe chronaxie changes, both from stimulation of the sympathetic and the dorsal roots, which are in the

opposite direction and which change upon decerebration of the cats. The experiments here are too few in number and three of the four experiments were done with intact circulation. Brücke and Krannich (1933) stimulated the 6–7L dorsal roots in cats under dial anesthesia and the 9–10 dorsal roots in frogs under urethane and measured chronaxie of the muscles of the limb. Their observations caused them to doubt the presence of any fibers in the dorsal roots of these forms which carry impulses altering the chronaxie of skeletal muscle.

Schneider (1929, 1930, 1931) has made a rather extensive investigation of a possible effect of the sympathetic nervous system in the skeletal muscle of the frog. He (1929) failed to confirm the observations of Ginetzinsky (1922) and Nakanishi, also those of Büttner and Heimbricht (1929). Furthermore, he (1931) has failed to find any difference in latent time and in the muscle action potentials during sympathetic stimulation.

Although Asher (1926) was inclined to doubt a sympathetic influence on skeletal muscle, he felt that further research was necessary to settle the matter. In the Physiological Institute at Bern, a number of important investigations have been made upon this subject by Maibach (1928), Labhart (1929), Charlet (1930), Michol (1930), Voser (1931), Haller (1932), Lehmann (1932) and Wenger (1933). This work has been reviewed from time to time by Asher, Charlet and Labhart (1929), and Asher (1931, 1932). This work confirms and extends considerably the observations of the Orbeli school. In general, they are inclined to interpret their observations to mean that the sympathetic effect may be attributed to a humoral mechanism, to the formation of adrenalin or sympathin which in turn acts upon the muscle. A similar explanation is invoked by Corkill and Tiegs (1933) and Wolff and Cattell (1934) for their observations on the effect of sympathetic stimulation upon the contraction of frog's muscle. The latter investigators also describe a decrease in the isometric contractions on stimulation of the dorsal roots in frogs.

Gasser (1930) has presented a critical discussion of the literature concerning contractures from indirect stimulation with the explanation of a possible humoral mechanism. Here we shall be concerned with briefly mentioning recent findings concerning the Sherrington phenomenon, a slow contracture of the skeletal muscles of the hind limb occurring after sensitization of the muscle by section and degeneration of its somatic motor fibers. This was first described by Sherrington (1894) and has been reinvestigated by Hinsey and Gasser (1928, 1930) and by Hinsey and Cutting (1933a, b). Hinsey and Gasser (1930) were tempted

to attribute both the dorsal root vasodilatation and the contracture of the muscle as due to antidromic conduction over sensory fibers, with the elaboration of a chemical substance to which the denervated skeletal muscle fibers had become sensitized. The Sherrington phenomenon has been one of the reasons for believing in a dorsal root parasympathetic innervation of muscle tonus because it has been thought that conduction over fibers arising in the dorsal root ganglia was responsible for this contracture. As Ranson (1929) emphasized, there is no observable effect upon normal mammalian muscle or its contractions to be attributed to dorsal root stimulation. Hinsey and Cutting (1933b) were able to show that when only sensory fibers were present in a motor nerve. after degeneration of the sympathetic and somatic motor components, no response was obtained; nor did they obtain any contractures by stimulating dorsal roots and their ganglia in preparations which had been sensitized by section and degeneration of their ventral root fibers. These observations seem to remove the Sherrington phenomenon as a support for dorsal root parasympathetic innervation of skeletal muscle. They demonstrated that the Sherrington contracture is due to impulses travelling over postganglionic sympathetic fibers and are inclined to explain it on a humoral basis with the elaboration of some sympathetic substance causing the sensitized skeletal muscle to contract. The paradox still remains that they failed to produce the contracture by stimulation of the preganglionic sympathetic fibers going to postganglionic neurons, whose axons are distributed to the extremity.

It is quite evident that impulses over sympathetic fibers have the ability to alter the function of skeletal muscle. No one has been able to demonstrate any contraction of normal skeletal muscle on stimulation of the thoracolumbar sympathetic fibers alone. It seems possible that the sympathetic effect on skeletal muscle could be indirect on a humoral basis instead of involving hypolemmal sympathetic endings which act directly on the skeletal muscle fiber. If the sympathetic vasomotor fibers cause the formation of sympathin (or a sympathetic substance or substances) in skeletal muscle, it may spill over and reach the skeletal muscle fibers either by way of the tissue fluids or the blood stream. The literature bearing upon possible humoral mechanisms in skeletal muscle is reviewed by Cobb and Wolff (1932) and Wolff and Cattell (1934) and will not be considered here. There are those who still believe that there are hypolemmal endings of sympathetic fibers in skeletal muscles. However the bulk of the anatomical evidence at the present time is not in keeping with the latter view.

Indeed postural contraction of skeletal muscle has not been shown to

depend on any other peripheral nervous substratum than is utilized by other types of contraction. The somatic motor fibers represent the efferent pathway to skeletal muscle fibers for all types of their contraction. Sherrington sums it up by saying, "Certainly the trend of experimental evidence is to show postural tonus to be simply part and parcel of ordinary motility; a manifestation of that ordinary rhythmic neuro-muscular activity by which are executed all our reflex and other acts involving skeletal musculature" (from Cobb and Wolff, 1932, p. 678).

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THE METABOLISM OF THE EVISCERATE PREPARATION

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The removal of the abdominal viscera in varying degrees has been performed for two main experimental purposes: 1, to study the metabolism of a preparation in which the predominant tissue is muscle, isolated from the visceral organs but with its physiological state otherwise as normal as possible; 2, to ascertain, from changes in the preparation following their removal, the nature of the metabolism of certain organs (liver, pancreas, etc.). It is inappropriate here to discuss in detail the vast literature relating to the latter purpose; evisceration experiments that have been performed to ascertain the functions of individual organs will be referred to only in so far as they throw light on the metabolism of the preparation that remains.

Except in a small number of experiments the eviscerate preparation has been employed in one of three forms: *anesthetised, decapitated,* or *decerebrated.* In order to obtain an anesthetised preparation as little removed from the normal as possible, the absence of effect on the blood sugar has been used as a criterion of suitability in selecting an anesthetic. That this criterion is not necessarily satisfactory has been made clear by the observation that amytal (if not all the anesthetics of the barbiturate group), while causing practically no hyperglycemia, has such a profound effect on the mechânism, or mechanisms, through which blood sugar is raised by asphyxia and by diabetic piqûre that these no longer cause hyperglycemia to the normal extent (31, 102). Decapitation and decerebration are useful means of avoiding continuous anesthesia. The former provides a quiet preparation in which slight variations in the level of the section are not attended by such differences in motor reaction as occur in the decerebrate animal. The fall in blood pressure that accompanies section below the medulla is, however, undesirable, as also, in some instances, is the necessity for artificial respiration. The term "decerebration" is used here not with the strict meaning given to it by Sherrington—"by a decerebrate preparation is understood one in which the whole brain in front of the posterior colliculi has been removed" (129)—but to mean a trans-section of the brain stem between

the cerebral hemispheres and the medulla. Appropriate decerebration leaves the nerve centres involved in muscle tone, blood pressure and respiration approximately normal. The decerebrate preparation is, however, more difficult to standardise on account of the changes which accompany slight variations in the level at which the brain is cut. In the cat, if the cut is made about the middle of the superior corpora quadrigemina and just posterior to the mammillary bodies, a relatively quiet preparation with normal muscular tone is obtained (75, 79); if it is made at a higher level, the movements of the animal may be disturbing, and if at a lower level, decerebrate rigidity appears (4, 5, 24, 50, 67, 79, 118, 128, 129). The position of the cut has also an effect on the behaviour of the blood sugar (34). In that its respiration is still controlled by changes within the preparation itself, the decerebrate animal is probably maintained in a more normal physiological condition than when a pump is employed.

THE FUNCTIONAL REMOVAL OF THE LIVER. Owing to the inaccessibility of the hepatic veins, the liver cannot easily be removed without obstructing the inferior vena cava. In experiments where the liver alone was removed this difficulty was skilfully surmounted by Mann (82, 83) who, by a modification of the method of Fischler and Schroeder (47) established in the dog a reverse Eck fistula, and later tied the inferior vena cava between the anastomosis and the liver. When a collateral circulation had developed, the portal vein was tied and the liver removed. Markowitz and Soskin (101) avoided the necessity of an Eck fistula and obtained satisfactory results by partial ligation of both the inferior vena cava and portal vein, thus causing development of the necessary collateral circulation. Drury and McMaster (36) applied this latter method to the rabbit.

In more acute experiments recourse has been had to other methods. Seegen (127), in an attempt to exclude the posterior part of the animal from the circulation, ligated the thoracic aorta and inferior vena cava above the diaphragm. Porges (115, 116) tied these vessels immediately below the diaphragm. Firor and Stinson described in detail (44) the successful removal of the liver of the dog in one operation by joining the portal vein to the inferior vena cava by means of a pyrex glass cannula and removing the liver with the attached piece of cava. They ascribe the success of the method largely to the use of pyrex glass in which blood will not readily clot. Firor and Eadie (43) applied the same method to the cat.

A technique, frequently adopted, was described by Pavy and Siau

(110): " . . . the rectum was divided between ligatures. The inferior and superior mesenteric arteries and the coeliac axis were ligatured and divided in turn, as were also the connections with the portal fissure of the liver. Stout ligatures were passed round the cardiac end of the stomach and afterwards the stomach, intestines, pancreas and spleen removed." By this method the liver, left in the animal, is deprived of its vascular inflow through the portal vein and hepatic artery, but retains its communication with the inferior vena cava through the hepatic veins. It has been suggested (7) that this method of evisceration does not remove all functional communication between the liver and the circulating blood, in that an addition of sugar to the blood may occur by an "ebb and flow" through the hepatic veins. An examination of the work on this problem leaves little doubt that the liver may still contribute to the composition of the blood. This was suspected by Pavy and Siau, who observed that, after evisceration, blood removed from the heart contained a quantity of sugar greater than normal. They investigated the matter by first performing their evisceration operation and later excising the greater part of the liver by ligating and cutting off the lobes. When this was done, the blood sugar fell, and following coma and what we now recognise as hypoglycemic convulsions, the animal died. Fischler and Grafe (46), discussing the possibility of a liver circulation in the dog with Eck fistula and ligated hepatic artery, discounted any circulation through the diaphragmatic vessels as being insignificant, but held that a slight reflux of blood through the hepatic veins lengthened the life of the preparation. Rich (120) investigated this problem more fully. He "tied securely the aorta just below the subclavian artery, the inferior vena cava just above the diaphragm, the right and left mammary arteries and veins within the thorax, the coeliac axis and abdominal aorta at that level, and besides, all the vessels entering the hilum of the liver. Wire ligatures were also placed about the rectus muscles and along the costal margin." He then injected Indian ink into the jugular vein and, after a few minutes, found it visible in the lobules of the liver; further, the ink found its way into parts of the aorta and mammary arteries beyond the ligatures. The abdominal aorta contained ink throughout its entire length, while the intercostal and diaphragmatic arteries stood out as black lines. When, in addition, the esophagus was ligated and cut across below the diaphragm, and the stomach, intestines, spleen and pancreas removed *in toto*, the ink, injected into the aorta just beyond the aortic valves, promptly appeared in the liver. The path of entry into the liver was thus traced to the

diaphragmatic vessels running along the sheath of the inferior vena cava and entering the liver in the region of the hepatic veins. That the phrenic artery is not a necessary pathway to these diaphragmatic vessels was shown by rapid blackening of the diaphragm and liver when ink was introduced by cannula into the aorta below the subclavian artery, the connecting vessels being the lower intercostal arteries. In addition, Rich demonstated that, despite ligatures on the inferior vena cava and aorta above the diaphragm and on the mammary arteries and veins, there still remained a venous return to the heart, since Indian ink introduced by burette into the liver through the portal vein, rapidly passed to the diaphragm and lungs. The complete exclusion of the liver from the circulation required, in addition to the tying of the hetic artery and portal vein, a ligature round the vena cava *between* the liver and the diaphragm.

There can be little doubt that the liver is not entirely excluded from the circulation by tying the hepatic artery and portal vein, and this was borne out in experiments by Peterson with the "functionally"[1] eviscerated cat (113). "Functional" evisceration terminated the hyperglycemia which follows section of the pons, but if the cutting of the pons was performed after evisceration, a temporary hyperglycemia resulted. A similar rise in blood sugar occurred when the functionally eviscerated preparation was made to rebreathe, or its liver was gently pressed by hand. No such rise resulted from pontine decerebration, or rebreathing, in three experiments in which the abdominal aorta and inferior vena cava were previously tied immediately below the diaphragm. It was observed that for a short time after the section of the pons, respiratory movements were usually exaggerated, and it seems probable that the temporary hyperglycemia produced in the "functionally" eviscerated preparation by this operation, or by rebreathing, may be due to movement of sugar-rich blood from the liver through the hepatic veins by the more extensive movements of the diaphragm. This is in accord with the view of Best, Dale, Hoet and Marks (7), and a similar conclusion has been arrived at by Mann and Magath (91).

Life-span. Abdominal evisceration is a drastic operation and, besides the loss of function which it is designed to achieve, the state of the resulting preparation varies greatly with the degree of exposure or handling of the tissues. In acute experiments, in which the liver has

[1] The term *functional*, though probably somewhat inappropriate, has been used to describe the method of evisceration described by Pavy and Siau, and, for the sake of uniformity, it will be employed here, but qualified by quotation marks.

been entirely removed or completely excluded from the circulation by the tying of the aorta and vena cava immediately below the diaphragm, the life of the preparaton has rarely been longer than four hours (14, 19, 43, 44, 78, 110, 115, 116). The immediate cause of death of the latter preparation which may occur, and probably most frequently occurs, before the blood sugar has become subnormal (113, protocols), is not clear, and the condition of the surgically hepatectomized animal resulting from hypoglycemia and terminating in death (e.g., 86) differs from that of the same preparation receiving glucose. Mann and Magath (89) have shown that with different species of animals the duration of life following hepatectomy is entirely dependent on the rate of disappearance of blood sugar. When, however, the hypoglycemia is relieved by injection of glucose, the liverless preparation survives longer (93). After Mann's three-stage operation—in which the greater part of the operative disturbance preceded, by some weeks, the actual removal of the liver, and no anesthetic was given after the final operation —5 to 8 hours elapsed before hypoglycemic symptoms appeared (89) and, with the injection of glucose, the animals lived from 18 to 30 hours (86) or longer (35). In this latter instance, according to Mann (86), the animal becomes restless, usually vomits, and its respiration is markedly increased without evidence of dyspnea or air hunger. Later it becomes ataxic, and after coma, sometimes lasting for hours, it dies, usually quietly and without other warning. The history of the decerebrate cat following "functional" evisceration is in many respects similar (2). Without receiving glucose, the preparation may live 8 or 10 hours or longer. During the later hours marked hyperpnea almost invariably occurs and sometimes death is unaccompanied by hypoglycemia.

The contrast between the completely hepatectomized and the "functionally" eviscerated animal, when neither receives glucose, may be explained on the ground that the liver in the latter instance is still supplying glucose to the blood. This would also appear true of the dog with Eck fistula and ligated hepatic artery which has been observed to live from 12 to 15 hours (51), 12 to 24 hours (102) and 5 to 24 hours (138). In these instances, however, death was preceded by convulsions. The protocols of experiments by Markowitz (99) show a rapid fall of blood sugar, together with prostration, in dogs with Eck fistula and ligated hepatic artery, but not with the same severity or with the same rapidity as after complete hepatectomy. Collens, Shelling and Byron (25) found that dogs, in which only the hepatic artery and its collaterals were cut, lived from 15 to 50 hours, the length of life depending on the existing store of glycogen.

The injection of glucose though postponing the death of the hepatectomized animal does so only for some hours (usually less than 48). This makes plain that hepatectomy is rapidly fatal through some other cause besides the loss of the supply of sugar to the blood. What this cause is, whether it be derangement of the metabolism of amino acids, accumulation of lactic acid in the blood, or some other change, has yet to be explained. When an Eck fistula is established and the hepatic artery is unobstructed, the life of the animal does not seem to be seriously impaired, but death may occur through feeding large quantities of protein either before or after the operation (45, 51).

Anderson *et al.* (2) correlated the life-span of the "functionally" eviscerated cat with the CO_2 content of its arterial blood and found that the rate at which this decreases is a reliable criterion of probable survival. The removal of blood for analysis, at a rate of about 2 cc. per hour contributed to earlier death (113), and despite the fact that some decerebrate eviscerate preparations lived over 10 hours, their condition, as judged by blood pressure, heart beat and minute-volume of expired air was comparable with that of the decerebrate but otherwise normal cat only for a period of less than 5 hours.

Oxygen consumption. a. *After evisceration.* There is much evidence to show that the oxygen consumption of an animal is diminished by removal of its abdominal viscera. Obstruction of the thoracic aorta and tying off all its branches above the point of obstruction, with the exception of the supply to one carotid which was joined to an external jugular vein, was observed by Bohr and Henriques (12) to diminish the oxygen consumption of the dog by only 30 per cent. They attributed the smallness of this diminution to the agency of a small collateral circulation through intercostal vessels, for, when this was blocked, the oxygen consumption fell markedly, to be further lowered by obstruction of the inferior vena cava. Bornstein (19) obtained a fall of from 34 to 70 per cent with an average of 56 per cent on "functional" evisceration of the dog under chloralose. Hunt and Bright (56), using the cat anesthetized with amytal, examined this question in some detail. They found that occlusion of the circulation to the stomach, intestines, pancreas and spleen (the hepatic artery being unobstructed) reduced the oxygen consumption by about 11.5 per cent, and that, when the liver was also removed, the figure rose to 41.5 per cent. For the decapitated and "functionally" eviscerated cat, Kilborn (62) quoted a fall of from 32 to 54 per cent within an hour of evisceration, and Ferguson, Irving and Plewes (42) observed a fall of about 35 per cent; in the urethaned and

"functionally" eviscerated cat, Evans, Tsai and Young (39) obtained, in four experiments, an average value of 37 per cent; and in the decerebrated and "functionally" eviscerated cat, Anderson *et al.* (2) found the fall to be 32 per cent.

Burn and Dale (20) showed that, in the decapitated and "functionally" eviscerated cat, the fall in blood sugar after the operation is accompanied by a decrease in the rate of oxygen consumption, even while the blood sugar is still above normal, and that, when the blood sugar is increased by injection of glucose, the oxygen consumption rises, while the R.Q. is not necessarily altered. Verzár and Fejér (135) observed that, in the diabetic animal, with relatively high blood sugar, not only did the injection of glucose cause an increase in R.Q. up till the fourth day, but that this was usually accompanied by increased oxygen consumption, which occurred even in some instances where the R.Q. failed to rise. According to Mann and Boothby (88) this rise is greater in the liverless animal than in the normal, and Markowitz (99) described the rise in the liverless dog as slow, its maximum being about 20 per cent above the value obtaining before injection. According to Soskin (132), this higher rate of oxygen consumption following glucose injection is maintained only for a short time and it is generally accepted to be due to a mass action effect of glucose—a carbohydrate plethora. Soskin (132) found that during the short survival of the abdominally eviscerated dog without glucose, its oxygen consumption was quite comparable with that of the same preparation receiving glucose, if the short initial effect of the carbohydrate plethora were neglected. Kil born's figures (62) for the oxygen consumption of the decapitated and "functionally" eviscerated cat progressively decrease. On averaging them, we obtain, for six animals with an average weight of 3.16 kgm., 206 cc. per kgm. of whole animal weight for the first hour, 180 for the second, and 107 for the third. These preparations were not receiving glucose, and for three only are blood sugar values recorded: the lowest of these, at the end of an experiment, is 0.079 per cent, which bears out the observation of Burn and Dale that the oxygen consumption of the decapitated and "functionally" eviscerated cat diminishes with falling blood sugar even while the latter is above normal. In the same preparation, Corkill, Dale and Marks (31) observed, toward the end of an experiment, a falling oxygen consumption with a falling blood pressure, but not to the same degree as recorded by Kilborn. Their preparations were, however, receiving glucose, and this may account for the difference. On the other hand, in six cats with an average weight of 3.04

kgm., Ferguson, Irving and Plewes (42) observed the oxygen consumption to decrease only from 227 to 207 cc. per kgm. an hour during the post-eviscerate period while no glucose was injected. Anderson *et al.* (2) using the decerebrated and "functionally" eviscerated cat observed an oxygen consumption of 220 cc. per kgm. per hour for a 2 kgm. animal receiving no glucose. The same value was obtained by Evans, Tsai and Young (39) with four urethaned, "functionally" eviscerated cats with an average weight for the whole animal of 2.26 kgm. Soskin (132) measured the oxygen consumption of six dogs, abdominally eviscerated with complete removal of the liver, unanesthetized and receiving glucose. His figures vary widely, ranging from 685 cc. for a 5.5 kgm. to 265 cc. for a 6.5 kgm. animal per kgm. per hour, the average oxygen consumption and weight being about 420 cc. and 6.5 kgm. In one experiment he recorded maximum and minimum values of 320 and 237 cc. for a 10 kgm. dog over a period of 5 to 6 hours.

In comparing absolute values of oxygen consumption for different individuals it must be remembered that, on a weight basis, the values for smaller animals are higher. The higher rate of oxygen consumption observed with the eviscerated and hepatectomized dog (99, 132) as compared with that of the eviscerated cat (2, 39, 42, 62) may be due to the fact that the former was unanesthetized while the latter was either anesthetized, decapitated or decerebrated.

A difference has been observed between the oxygen consumption of the decapitate and that of the decerebrate cat after "functional" evisceration. Almost without exception the recorded figures for the former preparation show a progressive decrease after evisceration. With the decerebrated and "functionally" eviscerated preparation, on the other hand, the oxygen consumption, after a period during which it remains fairly constant or falls slightly, has been observed to exhibit a terminal rise (2). Fischler and Grafe (46), who obtained a similar rise in the dog with Eck fistula and ligated hepatic artery, attributed it to the muscular activity involved in the convulsions that appeared before death. Anderson *et al.* (2) suggested that the increase might be due mainly to the muscular activity involved in increased respiratory movements, because of the close relationship which they found to exist between changes in oxygen consumption and in the minute-volume of expired air. Fischler and Grafe also observed a marked terminal increase in respiration. In the decapitate preparation the respiratory muscles are, of course, inactive.

b. *After hepatectomy.* Following operations designed to remove liver

function alone, somewhat varying figures for oxygen consumption have been obtained. Fischler and Grafe (46) tied the hepatic artery in the dog some weeks after an Eck fistula had been established. In four experiments the oxygen consumption, on tying the artery, fell by 30 to 50 per cent, the average being about 36 per cent. On complete hepatectomy of the rabbit by the method of Markowitz and Soskin (101), Drury and McMaster (36) found a diminution of only 8.3 per cent. For the frog, Cori and Buchwald (26) observed a fall of 15.6 per cent. Mann and Boothby (88) and Wilhelmj, Bollman and Mann (140) employed with dogs Kitchen's adaptation (64) of Boothby and Sandiford's method for respiratory analysis in man, and found no evidence of a fall in oxygen consumption after hepatectomy; the rate of oxygen consumption varied above and below the prehepatectomy level according to the degree of activity of the preparation. This finding by Mann and his colleagues contrasts with the results obtained by other methods.

From an examination of the figures obtained by Markowitz (99), it would appear that the oxygen consumption after hepatectomy is little different from that which occurs after removal of both pancreas and liver. His measurements were made on dogs in a respiratory cabinet. For three animals with an average weight of 3.9 kgm., one with its liver completely removed and the others with Eck fistula and ligated hepatic artery, the figures in cubic centimeters per kilogram per hour were 441, 515 and 463. For three others with average weight of 13.4 kgm., one with its pancreas and liver removed at the same operation and the others depancreatized two days before hepatectomy, the values were 451, 416 and 520. Somewhat lower values were obtained with animals from which the liver, stomach, intestines, pancreas and spleen were all removed, *viz.*, 360, 386 and 293, for animals averaging 10.3 kgm.

By themselves, these figures tell us little, but evidence from other sources (R.Q. measurements and blood sugar changes, yet to be considered) makes it probable that the metabolism of the liverless animal, during its short life, is unaffected by the removal of the pancreas, provided that pancreatectomy is performed at the same time as the removal of the liver.

THE RESPIRATORY QUOTIENT. a. *After evisceration.* The quotients observed after evisceration are almost always higher than the basal value for the normal animal.

Bohr and Henriques (12) measured the respiratory metabolism of the dog after cutting its brain stem and excluding from its circulation not only the abdominal viscera but also the greater part of its skeletal

musculature, and they obtained quotients which, with few exceptions, ranged from 0.9 to 1.26, with an average of about unity. Porges (115) and Porges and Salomon (116), with the "anterior preparation" of the rabbit and of the depancreatized dog, also obtained quotients near unity (0.902 to 1.19, with one exception), and offered the explanation that exclusion of the abdominal organs had deprived the animal of the power to oxidise fat and protein, and that the exchange of CO_2 and O_2 was consequently determined by the combustion of carbohydrate alone. The severity of the operation and the shortness of the life of the "anterior preparation" were advanced in criticism of the value of these experiments, and high quotients observed with the same preparation (124), and with the dog with Eck fistula and ligated hepatic artery (46), were attributed to loss of CO_2 through diminution of the alkali reserve, and not to any change in oxidation. Porges' interpretation of his results was later challenged by Murlin, Edelmann and Kramer (107), who believed that a loss of CO_2 from the tissues of the body produced by means other than oxidation might explain the observed rise in R.Q. Their criticism was based on the results of experiments on dogs, anesthetized with chloretone, in which it was observed that clamping of the abdominal aorta and inferior vena cava was followed in almost every instance by heightened R.Q., lowered blood CO_2, and increased ventilation. When the R.Q. did not rise, the blood CO_2 did not fall, and inone experiment in which the blood CO_2 rose somewhat, the R.Q. fell.

In more recent work, involving the "functionally" eviscerated preparation, support has been given to both sides of this problem. Burn and Dale (20), in studying the action of insulin in the decapitated and "functionally" eviscerated cat, observed quotients so near unity as to suggest strongly that this rise is due to a process for which only a quotient of unity is possible. This was borne out by the later work of Corkill, Dale and Marks (31). Kilborn (62), under the direction of Macleod, examined this question and supported the finding of Murlin, Edelmann and Kramer. In experiments with the decapitated and "functionally" eviscerated cat, he demonstrated that a heightened R.Q. was associated with loss of blood CO_2, which could readily be brought about by overventilation, and that when the CO_2 of the blood was maintained at its normal level, the R.Q. after evisceration was not necessarily different from that before it, while, on the other hand, excessive ventilation could raise the R.Q. to a value greater than unity. Bornstein (19) has supported this contention in his work on dogs anesthetized by chloralose and "functionally" eviscerated or partially hepatectomized as well. When

glucose was not injected, he obtained quotients varying from 0.72 to 0.9, accompanied by a marked fall in oxygen consumption. He attributed higher quotients to elimination of CO_2 from the tissues as a result of the increased ventilation that followed evisceration. On injecting glucose he obtained quotients of unity and even higher. This readiness with which over-ventilation can remove CO_2 from an experimental animal has been referred to by Krogh (66), and from man, by Dautrebande (32).

Kilborn, Soskin and Thomas (63) demonstrated that with this diminution in blood CO_2 there is a concurrent proportional increase in blood lactate. Ferguson, Irving and Plewes (42, 57) and Irving and Foster (58) confirmed the results of Kilborn and showed that during over-ventilation, both the blood and muscles lose CO_2 but not in sufficient quantity to account for the height to which the R.Q. rises. Irving and Foster have expressed their belief that the true R.Q. of the eviscerate cat is about 0.8. With the decerebrated and "functionally" eviscerated cat Anderson *et al.* (2) also obtained results in keeping with the view that the true R.Q. of this preparation is less than unity. The R.Q. with slightly falling blood CO_2 was 0.85 or less, and when the blood CO_2 fell rapidly the quotient rose, sometimes to unity or higher. A high quotient and falling blood CO_2 content was accompanied by increasing blood lactate and by hyperpnea. Peterson (114), from an examination of the figures from six such experiments, found that the relationship of the changes in blood lactate and blood CO_2 is linear, except when the blood CO_2 is below a certain value; and further, that this relationship is quantitatively similar to that which exists between the lactate formed and the CO_2 displaced in the reaction:

$$CH_3 \cdot CHOH \cdot COOH + NaHCO_3 \rightarrow CH_3 \cdot CHOH \cdot COONa + H_2O + CO_2$$

He also found a probable linear relationship between the R.Q. and the *rate* at which the blood CO_2 falls such that when the latter is zero the R.Q. is about 0.8. He compared the quantity of CO_2 lost from the blood with that given out, at the same time, in the expired air to raise the R.Q. from this value to the observed value, and showed that the process can be explained if a mass of tissue equal to 48 per cent of the body weight is losing CO_2 at the same rate as is the blood. The rate of diminution of the total CO_2 content of the muscles of the decapitated and "functionally" eviscerated cat, measured by Ferguson, Irving and Plewes and by Irving and Foster, is not sufficiently great to justify the assumption that the CO_2 store of the muscles is the source of this extra CO_2 output.

b. *After hepatectomy.* There is no evidence that the removal of the pancreas as well as the liver results in a respiratory quotient in any way different from that which obtains after hepatectomy alone, unless the removal of the pancreas precedes hepatectomy by some days. There is convincing evidence that after pancreatectomy alone the basal R.Q. is depressed (32, 41, 60, 133, 134, 135, 139), and Hédon (52) has stated that there is a gradual fall attaining approximately the fat level in four days. Secondly, it has been shown that the injection of glucose, in complete diabetes, no longer raises the R.Q. (6, 21, 49, 60, 99, 109, 117, 123, 134, 135, 139). Verzár and Fejér (136) have observed that this loss is also a gradual one becoming complete about the fourth day after pancreatectomy. The diabetic condition appears to develop more slowly than do the severe metabolic disturbances that follow removal of the liver, hence it is to be questioned whether in the short life of the hepatectomized animal the simultaneous removal of the pancreas is of any significance in determining the nature of the metabolism. Mann and Magath (96) observed that after total hepatectomy the R.Q. invariably rose, but as the sugar concentration of the blood fell, the R.Q. also fell and in at least one instance fell to its normal level. The injection of glucose caused an immediate rise in the quotient.

Soskin (132) records quotients for the eviscerated and surgically hepatectomized dog receiving glucose which have an average value of 0.94. Following hepatectomy alone he obtained in two experiments values ranging from 0.93 to 1.13, and after pancreatectomy alone, performed some days previously, 0.669 to 0.718.

Markowitz (99) obtained a quotient of 0.87 following complete hepatectomy in the dog. Injection of glucose raised the quotient to 0.99 which was maintained for two hours. In an experiment in which the pancreatectomy preceded the removal of the liver by about two days the quotient was again about 0.87 but it then fell when glucose was injected. When the dogs were eviscerated with complete removal of the liver the quotient was higher (between 0.89 and 0.99) and showed little change on glucose injection. Mann and Boothby (88) obtained with the dog by the method described by Kitchen (64) a basal fasting R.Q. of about 0.7 to 0.75, which, following hepatectomy, was elevated but not to unity (0.85 to 0.95). There was always a rise in the quotient on injecting 0.25 gram or more of glucose per kilogram of animal weight.

c. *The significance of the observed respiratory quotient.* There seems little doubt that the true R.Q. of the eviscerated animal is much below unity. Whether or not it is the same as that of the intact animal is

another question. Almost without exception some degree of rise above the pre-evisceration value has been observed, which is interpreted by some authorities as indicating that a process for which the quotient is low, *viz.*, gluconeogenesis, normally takes place in the liver. The significance of the respiratory quotient in relation to the qualitative nature of the metabolism has already been thoroughly explored and amply reviewed (Rapport, 119; Richardson, 122), and it will suffice here to point out that two contrasting interpretations have been put on the low quotient which obtains after pancreatectomy: 1, that the tissues are no longer able to oxidize cabohydrate; 2, that the formation of new sugar from fat is increased while sugar is still being burned. Indeed, as Rapport has clearly pointed out, the assumption that there is still combustion of carbohydrate is essential to acceptance of this second view, otherwise the quotient in diabetes would necessarily be lower than it actually is. The difficulty in reconciling these two views can be inferred from the relatively recent writings of two authorities: "One by one the bulwarks of the doctrine of the conversion of fat into glucose have been shattered and it may now be relegated to the realm of scientific superstition" (Lusk). "Thus one more nail is put into the coffin of the long moribund and now quite dead theory that diabetes is due to the loss of the power of the tissues to oxidise carbohydrate. The power may be depressed . . . ." (Macleod).

There is nothing to be gained at present by reviewing again the available evidence for these two views. The decision as to which is correct awaits further investigation.

BLOOD SUGAR AND MUSCLE GLYCOGEN. The fall of blood sugar which follows abdominal evisceration is undoubtedly due to the removal of the liver. This fact was demonstrated by Bock and Hoffmann (10) and after frequent confirmation it has been clearly established by the detailed work of Mann and his colleagues (86).

The similarity in regard to oxygen consumption and R.Q. which exists between the hepatectomized animal and that from which both the liver and pancreas is removed is paralleled by the behaviour of the blood sugar in these two preparations. Kaufmann (61) found that the exclusion of the liver from the circulation resulted in a fall in blood sugar in the diabetic as well as in the normal dog, and Macleod and Pearce (74) have demonstrated that the rate of decrease of blood sugar in the two instances is similar. This was confirmed by Mann and Magath (94) who showed that if the pancreas be removed some time prior to hepatectomy this latter operation still results in a rapid decrease in

blood sugar but the symptoms characteristic of hypoglycemia now appear at a higher blood sugar level. Burn and Dale (20) and subsequently others, have demonstrated that insulin still lowers the blood sugar after "functional" evisceration. The confirmation of this fact using the surgically hepatectomized dog (87, 95) removes any question of the observed hypoglycemic action being dependent on a partial circulation through the liver. Burn and Dale's observation that the fall in blood sugar following insulin injection in the eviscerate preparation is accompanied by an increase in oxygen consumption and in the glycogen content of the muscles has been confirmed with the intact rat (27).

The possible quantitative relationship existing between the carbohydrate which disappears as glucose from the blood and that which can be accounted for by increased muscle glycogen *plus* the carbohydrate equivalent of the oxygen used, has been examined by Best, Dale, Hoet and Marks (7) with the "functionally" eviscerated cat and by Cori and Cori with the intact and adrenalectomized rat. This matter has already been fully discussed by Cori (29) and by Macleod (72) and need not be dealt with here.

The observed relationship of blood sugar to muscle glycogen on the one hand (16, 131) and to liver glycogen and gluconeogenesis on the other (14) is such as to justify the view that the liver is the sole source of blood sugar. The falling blood sugar which follows hepatectomy is associated with the absence of the glycogen of the liver and death may occur from hypoglycemia while the muscles are still rich in this substance. Further, the hyperglycemic action of adrenaline (22, 98, 131), ether anesthesia (131), asphyxia (73) and of piqûre (11, 113, 125) are all inhibited by the removal of the liver.

Cori (28) has discerned in the changes which glucose can undergo in the body, a probable cycle of events in which the glucose of the blood is convertible to muscle glycogen through the agency of insulin (8, 22, 30, 100), while the reverse process does not take place; instead, the muscle glycogen, especially when adrenaline is injected, gives rise to blood lactic acid (29, 48, 53, 69, 104) which in turn is converted to glycogen in the liver (28, 69, 104) and the cycle is completed by the formation of blood sugar from liver glycogen. The evidence for such a cycle is convincing, and applied to the eviscerate preparation, the process

blood sugar → muscle glycogen → blood lactic acid

is of special interest. The importance of insulin for the formation of muscle glycogen from blood sugar has been emphasized by Debois (33),

who attributes the failure of glycogen to be restored in the muscles after it has diminished during muscular contraction, to the removal of the pancreas or even to the cutting of the vagi. He believes that the integrity of the latter is necessary for the normal secretion of insulin. This result, in acute experiments, contrasts with the slow onset of other sequelae of pancreatectomy, e.g., changes in R.Q. (51), and calorigenic response to glucose injection (99, 135); besides, there is no record of a lack of insulin in the vagotomized but otherwise normal animal. In addition to emphasizing the importance of insulin for the formation of liver glycogen from blood sugar this result suggests that the breakdown of muscle glycogen, presumably to lactic acid, is an irreversible one. In presence of the liver, lactic acid formed from muscle glycogen might be carried to the liver, there converted to glycogen, and so made unavailable for resynthesis of muscle glycogen, but this reasoning does not apply to the eviscerated preparation. Peserico (112) found, on the other hand, that during a period of rest following contraction of the gastrocnemius muscle of the depancreatized dog, the glycogen of the muscles became restored as in the normal animal. Eggleton and Evans (37) observed a recovery of muscle glycogen during rest after contraction of the limb muscles of the "functionally" eviscerated cat under luminal. Hoet and Ernould (54) agree that such a recovery of muscle glycogen does occur, but ascribe it to the agency of insulin in the blood. They hold that excess of this hormone is discharged from the pancreas during evisceration unless this is performed by tying the portal vein before obstructing the arterial inflow to the viscera. Cleghorn and Peterson (23) found that there is a recovery of glycogen in the skeletal muscles of the decerebrated and "functionally" eviscerated cat even when the experiment was performed twenty-four hours after removal of the pancreas, and they are of the opinion that the loss of blood along with the removed viscera by the method of evisceration recommended by Hoet and Ernould would be such as to result in a blood pressure so low that the restoration of glycogen, which they observed in their own experiments, would not occur.

It seems probable that muscle glycogen can be formed in the eviscerate preparation from lactic acid or some product of glycogen breakdown which cannot be estimated as such by the Pflüger method. The blood lactic acid of the "functionally" eviscerated cat has been observed (113) to rise by more than 150 mgm. per 100 cc. during severe muscle contraction and to fall immediately on cessation of stimulation, attaining its original value in less than an hour. It has been demonstrated that in

the "functionally" eviscerated animal a high blood lactic acid concentration, occasioned by anesthesia or operation, will likewise fall (37).

As an alternative to the view that lactic acid can be converted to muscle glycogen in the intact muscles of the eviscerate preparation, some other explanation of its disappearance from the blood stream is needed. It may be that it simply diffuses into other tissues in which its concentration is lower, but it cannot diffuse into the skeletal muscles in which it is formed, as the tension of lactic acid within them at the end of the contraction period must be as high as that of the blood. At the same time, unless there is a difference in the qualitative nature of the metabolism of the two tissues, it is difficult to reconcile the findings of Meyerhof regarding the conversion of lactic acid to glycogen in the excised muscle of the frog with an absence of this power in the muscles of the eviscerated mammal. At least two questions still remain to be answered before we can state with any degree of certainty that the observed recovery of muscle glycogen is not the result of insulin action and that the lactic acid which disappears from the blood of the "functionally" eviscerated preparation is not converted to liver glycogen. These are: Is the progressive onset of diabetic symptoms, observed during the four days immediately following pancreatectomy, due to a slow disappearance of insulin? and Is there in the "functionally" eviscerated animal a sufficient circulation in the liver to bring about the formation of liver glycogen from lactic acid?

Although a sudden increase in the blood lactic acid of the eviscerate preparation through anesthesia, or severe muscle stimulation is only temporary if the exciting cause is removed, a slow rise constantly occurs in the decerebrated and "functionally" eviscerated preparation at rest (2). The part played by the liver in the cycle of carbohydrate described by Cori offers a possible explanation of this. The muscles of respiration will presumably form lactic acid during their activity and if the normal path of its removal is blocked through the absence of the liver, lactate will accumulate in the blood and tissues with a depletion of the alkali reserve. On the other hand, Evans *et al.* (40) have questioned the view that the lactic acid of the blood is derived from muscles except in special circumstances (*e.g.*, severe exercise). They found that the lactic acid which accumulated in defibrinated blood pumped round a circuit containing an oxygenator could be explained as resulting from glycolysis. Its rate of formation and the rate of disappearance of glucose were slowed by a reduction in pH below 7.4. Further, with the heart-lung preparation, they confirmed the finding of McGinty (71)

that the heart removes lactic acid from the circulating defibrinated blood, and that the difference between the rate of lactic acid removal and the estimated rate of its formation by glycolysis agreed closely with the change in its concentration in the blood which they observed to occur. They hold the view that blood lactic acid, when its production by the muscles is not excessive, results from glycolysis and is constantly removed by the liver, the heart and probably the muscles.

PROTEIN AND PURINE METABOLISM. The extensive literature relating to the part played by the liver in the formation of urea, ammonia and uric acid has been reviewed in a comprehensive manner by Mann (86). That the liver plays a major part in urea formation is no longer questioned; indeed there is no evidence that this substance can be formed from ammonia in any other organ in the mammalian body. The presence of arginase in kidney, intestine and muscle (65) may account for a smaller extra-hepatic formation of urea.

Following hepatectomy, the urea of the blood and other tissues decreases while the amino-nitrogen and ammonia of the blood, tissues and urine increase. The decrease in blood urea can be prevented by removal of the kidneys (13). There is evidence that the ammonia which appears in the urine during acidosis is formed in the kidney (108), but the observation (17) that removal of the liver results in an increase in the ammonia of the blood and other tissues, while ammonia introduced into the blood stream in the form of ammonium salts appears in the urine unchanged, shows that ammonia formation does not occur entirely in the process of excretion. This increased excretion of ammonia following hepatectomy is of interest in relation to the progressive acidosis that has been observed to occur in the eviscerate preparation (63). Its production, however, would appear to be dependent on a supply of urea, for, according to Bollman and Mann (17), when the concentration of urea in the blood following hepatectomy has reached a minimal value, the excretion of ammonia diminishes, and can be restored by intravenous injection of ammonium salts or urea, but not of amino acids, uric acid or creatinine. Experiments relating to the part played by the liver in the formation or conversion of uric acid have been carried out mainly with birds and dogs. In the bird, removal of the liver is followed by a decrease in the excretion of uric acid and an increase in that of ammonium lactate (104). It is believed that the liver is the site of the formation of uric acid from urea which takes place normally in the bird in company with a smaller extra-hepatic supply from nucleoprotein (55, 70). In the dog, however, the establishing of an Eck fistula or removal of the

liver causes an increase in the uric acid content of the urine (15, 51, 90, 111) and of the blood as well (15, 90, 111). The rate at which the acid accumulates in the blood is increased if the kidneys are removed with the liver (15, 109). The rate of uric acid excretion has been found to be proportional to the decrease in the allantoin excretion (1, 18). It appears that while the removal of the liver inhibits the production of uric acid from urea in the bird and of allantoin from uric acid in the dog, it does not prevent the formation of uric acid from nucleoprotein.

BILE FORMATION. The part played by the liver in the formation of bile has been the subject of much work that has already been reviewed (77, 85, 121, 126, 137). Earlier observations with hepatectomized birds (76, 106, 138) were to the effect that no formation of bile pigment occurred after liver removal. This view has had some support from work on mammals but in the main it has been denied (14, 59, 81, 90, 139 and others), and there is evidence that bilirubin can be formed in cells of the reticulo-endothelial system and is probably formed in this system whenever it occurs in the body (3, 9, 38, 69, 76). On the other hand, the epithelial cells of the liver do not appear necessary for its formation. Bollmann, Mann and Magath (14) found that bilirubin appeared in surgically hepatectomized dogs that lived for more than six hours after the operation. The length of this period necessary for the production of the pigment in observable quantity probably explains why it was not found in the majority of experiments with the short-lived "anterior preparation" used by McNee and Prusik (78). The conclusion come to by Mann, Sheard *et al.* (97) from spectrophotometric analysis of blood, that a much greater quantity of bilirubin is formed in the red bone marrow than in either the spleen or the liver, has been questioned by Matthieu (103), who adduces evidence to show that bilirubin is not the only substance involved in these estimations.

Whether the part played by the liver in the formation of bilirubin be large or small, it is agreed that the pigment is eliminated from the body mainly, if not solely, through this organ; and, as observed by Mann and Magath (90), bilirubin formed in the animal body after removal of the liver accumulates in large part in the tissues, especially in the fatty tissues.

Evidence as to the site where the bile salts are formed is altogether too scanty to justify the postulate (130) that they are formed in the liver; and, in the absence of a satisfactory method for their quantitative estimation, we have no means of deciding whether in the eviscerate animal these substances are absent, or present in increased amount.

Mann (84) has estimated the cholesterol content of the dog before and at various times after hepatectomy and has found no significant changes in its concentration.

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